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THE BOTANICAL REVIEW

VOL. XVII JUNE, 1951 No. 6

ANTIBIOTICS PRODUCED BY FUNGI

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INTRODUCTION

During the past decade many thousands of fungi have been passed through routine screening tests to determine whether they produce antibiotics. Unfortunately the screening tests have often been limited in scope, antibiotics toxic to specific pathogenic bacteria being generally sought, so that a full appreciation of antibiotic potentialities has not been obtained. Unfortunately, too, in many cases, perhaps in the majority of them, the fungi screened have not been identified, and consequently many results have not been published. Nevertheless published results of screening tests and of actual isolation of antibiotics are now sufficiently numerous to make it possible seriously to attempt to answer the two following general questions: Is the capacity to produce antibiotics evenly spread over all taxonomic groups of fungi? Is there a tendency for related fungi to produce similar antibiotics, and, contrarily, is there a tendency for fungi in distinct taxonomic groups to produce distinct antibiotics? These questions have a practical importance, since, unless they can be answered, it is impossible to assess the chances of finding new antibiotics by testing species as yet untested. Furthermore, as will be seen from the following pages, a survey of antibiotic production in the various taxonomic groups of fungi serves to show which groups have received adequate attention and which have been neglected.

In the first section of this review, records of antibiotic production in each of the taxonomic groups of fungi are considered in turn, account being taken of negative results as well as of positive indications of antibiotic production. In the second section, brief characterisations of the isolated antibiotics are given, based on

their more important physical, chemical and biological properties. In doing this, a fairly complete survey of the literature has been made, and it is believed that account has been taken of most records of screening tests and of other reported demonstrations of antibiotic production, including actual isolations of antibiotics in crude or pure form. The literature references given are not complete, since thousands of papers have been published in this field. In particular, penicillin, which merits individual treatment, if only because research on its production and properties is in volume comparable to that on all other fungal antibiotics combined, receives only passing mention in this review. Nevertheless the bibliography should suffice to give access to the complete literature without undue difficulty.

DISTRIBUTION OF THE POWER TO PRODUCE ANTIBIOTICS IN TAXONOMIC GROUPS OF FUNGI

The numerical data in this section are based on what is believed to be an almost complete survey of the literature. The majority of the records come from a relatively small number of papers reporting the results of screening tests (14, 56, 82, 113, 119, 120, 133, 168, 216, 249–262). To these have been added the reported results of more intensive study of single fungi; since studies of the latter kind have on occasion arisen from the results of unpublished screening tests, the numerical data may be somewhat biassed, indicating a greater frequency of antibiotic-producing fungi than is really the case. It is believed that this bias is not great. Some attempt has been made to indicate what proportion of the known species in each group has been tested for antibiotic production, using for this purpose the estimations of Ainsworth and Bisby (2).

It should be recognized that many different methods of test have been used and that for this reason, and as a result of strain specificity, different workers have obtained different results from what purports to be the same species. Thus in the data which follow, an "inactive" species is one which has been tested but has not been found on any occasion to produce an antibiotic, and an "active" species is one which has been found by at least one worker to produce an antibiotic.

The data on which the present review has been based indicate that of 245 genera tested, including a total of 2191 species, 120

genera contain active species, the total number of such active species found being 785. The distribution of these among the main divisions of fungi is shown in Table I. It will be seen that the Basidiomycetes and Fungi Imperfecti have been the most fruitful groups in the search for antibiotic-producing fungi, but this may be only a reflection of the fact that it is these two groups which have been most intensively studied.

TABLE I

	Phycomycetes	Ascomycetes	Fungi Imperfecti	Basidiomycetes
No. of genera tested	15	49	44	137
No. of genera active	2	16	20	82
No. of species tested	28	86	412	1,665
No. of species active	2	24	189	570

It must be recalled at this point that a high proportion of these data has been obtained by tests which involve observations of antagonism in agar culture between the fungus under investigation and various test organisms. It is not always possible to confirm the production of an antibiotic when experiments in agar are followed by experiments using cultures on liquid media. Robbins, Kavanagh and Hervey (217) have suggested that some of the positive results seen in agar culture are due to local changes in hydrogen ion concentration; in some experiments of theirs with species of *Polyporus, Fomes* and *Poria,* they considered that as many as 50% of the apparently active fungi owed their activity to local production of acid. The data in Table I might thus be expected to give a somewhat exaggerated impression of the numbers of fungi which produce antibiotics; in fact, the tendency of agar antagonism tests to give false positive results is probably counterbalanced by the tendency of these tests to give false negative results where the antibiotic produced is insoluble in water or of high molecular weight.

PHYCOMYCETES

Only two of the 28 species of Phycomycetes examined have given any indication of antibiotic activity, and in neither case has the active material been isolated. Wilkins and Harris (259) have reported that *Phytophthora erythroseptica* is active against *Bact.*

coli and *Staph. aureus*. Harris (129) has reported that a strain of *Mucor racemosus* produces, in liquid culture, a thermostable substances toxic to *Sarcina lutea* and *Serratia marcescens*.

There are 235 genera with about 1050 species in this group (2), and more species need to be examined before one can safely conclude that antibiotic production by Phycomycetes is rare. It is true, on the other hand, that, in spite of detailed biochemical investigation of quite a number of species, all the known extracellular metabolic products of Phycomycetes are relatively simple aliphatic substances, such as ethanol, acetaldehyde and carboxylic acids of low molecular weight; it may be that as a group they are not capable of synthesising the complex aromatic molecules characteristic of so many antibiotics.

ASCOMYCETES

The 49 genera and 86 species of this group which have been tested fall into seven of the 15 orders recognised by Martin (167). No species have been tested from the following orders: Taphrinales, Myriangiales, Dothidiales, Hemisphaeriales, Erysiphales, Laboulbeniales, Hysteriales, Phacidiales. In one of these orders (Erysiphales) the species are obligate parasites so that it is not possible at present to test them; nevertheless the seven remaining orders account for 542 genera and 3740 species which may contain fertile ground for investigation. The orders containing species which have been examined are considered below.

ENDOMYCETALES. Nine species from six genera (*Ashbya, Byssochlamys, Eremascus, Eremothecium, Hansenula, Saccharomyces*) have been examined but none has shown activity. This order contains 43 genera and 135 species.

EUROTIALES. If the ascosporic species of *Penicillium* and *Aspergillus* are excluded, this order contains about 100 species in 48 genera; five species, falling in three genera (*Elaphomyces, Gymnoascus, Monascus*), have been tested, and one has been found to be active. Karow and Foster (145) reported that patulin is produced by a species of *Gymnoascus;* the possession of a *Penicillium*-like imperfect stage by some species of *Gymnoascus* should be remembered in this connection.

HYPOCREALES. Nine species from four genera have been tested, and five species, including representatives of all four genera tested

(*Cordyceps, Gibberella, Neocosmospora, Melanospora*), have been found to be active. This order contains 125 genera and 800 species. The active species described are *Cordyceps capitata* and *C. militaris,* both of which produce antibacterial substances (252); *Neocosmospora vasinfecta* which produces a substance toxic to *Staph. aureus* but not to Gram-negative bacteria (259); *Melanospora destruens,* found by Cook and Lacey (82) to produce an antibacterial substance in liquid media; and *Gibberella fujikuroi.* From the latter species Yasue (275) has isolated a generally antibacterial substance which he has named "fusaric acid", but no antibiotic has actually been isolated from the other species mentioned.

Trichoderma viride (see Fungi Imperfecti), which is known to produce two antibiotics (gliotoxin and viridin), is said (34) to be the imperfect stage of *Hypocrea rufa* which falls in this order. Most species of *Gibberella* have a *Fusarium* imperfect stage, and many species of *Fusarium* (see below) have been shown to produce antibiotics.

SPHAERIALES. This order is the largest in the Ascomycetes, comprising 398 genera and about 4350 species. Of 31 species from 14 genera examined, seven from three genera have been found to be active. Of 17 species of *Ceratostomella* examined by Robbins et al. (249), *C. rostrocylindrica* and *C. ulmi* showed antibacterial activity not attributed to acid. *Ustulina zonata* (254) is weakly antibacterial. Four species of *Chaetomium* have been shown to produce antibacterial substances—*C. cochlioides, C. convolutum, C. spirale* and one undetermined species. From *C. cochlioides* and *C. spirale* an antibiotic named "chetomin" has been obtained (117, 118, 233).

HELOTIALES. From this order, which contains 268 genera and 1400 species, only nine species from eight genera have been tested. Of these, three species—*Dascyscypha luteola* (252), *Sclerotinia minor* (259), *Spathularia clavata* (252)—have given some indication of producing antibacterial substances, but none has been isolated.

PEZIZALES. This order is said to contain 56 genera and about 500 species. From tests of 22 species from 13 genera, seven species from four genera have been found to be active. *Acetabula vulgaris, Aleuria subrepanda* and *A. vesiculosa, Bulgaria inqui-*

nans, Galactinia badia, G. succosa and *Morchella esculenta* have been shown to produce antibacterial substances (252), but, again, none has been isolated.

TUBERALES. This order contains 28 genera and 140 species. Apparently only one species—*Tuber melanosporum*—has been tested. Chaze (75) has found an antibacterial substance in the mycelium of this truffle growing in liquid culture and also in the culture filtrate.

The general impression gained from this survey of antibiotic production in the Ascomycetes is that there is an immense field for further investigation, not only in those mentioned orders in which no species has yet been examined but also in those orders which have received some attention. As yet, apparently, only 86 species of the 12,000 or more in the group have been examined. Moreover, though evidence is available that 24 species of the 86 examined produce antibiotic substances, only three antibiotics (patulin, fusaric acid and chetomin) have been isolated.

FUNGI IMPERFECTI

This difficult group is thought to contain 1252 genera and some 10,500 species. Ainsworth and Bisby (2) suggest that possibly one-third of the species have named perfect stages (mostly Ascomycetes), possibly another third have perfect stages not yet determined, and the rest no perfect stage. Of this great number of species, about 412 species from 44 genera have been tested for antibiotic activity. This estimate of the number of species tested is probably exaggerated, owing to the use of many synonyms in the genera *Penicillium* and *Aspergillus*. Nearly 75% of these 412 species are from three genera, *Aspergillus, Fusarium* and *Penicillium*. In all, 188 species from 20 genera have been reported to be active; if account is taken of synonymy the number of active species would probably be reduced to 157.

Martin (167) divides the Fungi Imperfecti into three form-orders. These are considered in turn below; it will be seen that they have been very unevenly investigated.

SPHAEROPSIDALES. This form-order comprises at least 505 genera and 5200 species. Apparently only six species from five genera (*Ascochyta, Diplodia, Nematospora, Phoma, Septoria*) have been examined. Two species have been found to produce anti-

biotics; Cercos (70) has shown that *Septoria nodorum* produces a substance toxic to a number of Gram-positive bacteria but not to several Gram-negative bacteria, and Kent (152) has demonstrated the production of an antifungal substance by *Diplodia zeae*. Neither antibiotic has been isolated.

MELANCONIALES. No record can be found in the literature that any species has been tested from this form-order which contains about 1000 species in 86 genera.

MONILIALES. This form-order comprises about 4100 species in 640 genera. Of these, 405 species from 39 genera have apparently been tested, 213 species from 16 genera being active. Most of the active species, as will be seen, come from the genera *Aspergillus, Fusarium* and *Penicillium*. A great number of antibiotics from this group of fungi have been isolated in pure form. The genera containing active species are as follows:

Alternaria. Some strains of *A. porri* and *A. solani* produce an antifungal substance. From *A. solani* the substance has been isolated in pure form and named "alternaric acid" (53). This material is particularly interesting in that it seems probable that its production in the host plant is responsible for some of the symptoms of the diseases of plants caused by *A. solani*. *Alternaria citri* produces an antibacterial substance when grown in liquid culture (102), but this has not been isolated.

Aspergillus. This genus has been divided by Thom and Raper (227) into 14 groups. It has been intensively studied, and only three species (*A. miyakoensis, A. montivedensis, A. delacroixii*) have not been tested. Production of a considerable number of antibiotics by species of *Aspergillus* is now established, and the numerous records will best be understood if we consider each group in turn.

Aspergillus clavatus group

This group comprises two species, *A. clavatus* and *A. giganteus*. Strains of both species have frequently been found to produce the antibiotic patulin (23, 105, 140, 148, 235, 248). Some strains of *A. giganteus* produce a penicillin (196).

Aspergillus glaucus group

This is one of the largest groups of Aspergilli, comprising 19 species. Many organisms from this group have been tested (102,

113, 119, 258, 261), and production of antibacterial substances by *A. chevalieri* (119), *A. echinulatus* (113), *A. glaucus* (261), *A. gracilis* (119), *A. mangini* (119), *A. medius* (258), *A. repens* (261) and *A. restrictus* (261) has been reported. In no case has the antibiotic been isolated; in some cases, but not all, it seems probable that the antibiotic concerned is a penicillin.

Aspergillus fumigatus group

Many workers have reported (6, 8, 73, 171, 184, 236, 258, 261) that *A. fumigatus* produces antibacterial substances. Four substances seem to be involved; spinulosin (6, 8, 184), fumigatin (6, 184), gliotoxin (171) and helvolic acid (fumigacin) (73, 171, 236). Of these, gliotoxin and helvolic acid are the most actively antibacterial. No antibiotic activity has been reported for *A. fischeri,* the only other species in this group.

Aspergillus nidulans group

The production of a substance, active only against Gram-positive bacteria, which is probably a penicillin, is general in this group and has been reported for *A. caespitosus* (120), *A. nidulans* (119, 258), *A. quadrilineatus* (120) and *A. unguis* (102). In addition, another weakly antibacterial substance has been isolated in partially purified form (102) from *Aspergillus caespitosus.*

Aspergillus ustus group

Kurung (157) first reported that *A. ustus* produced an antibiotic inhibitory to *Mycobacterium tuberculosis,* and succeeded in isolating it in crude form. Since then a variety of substances with antibiotic activity have been isolated including ustin (93), which contains chlorine, aun_1, aun_2, aun_3 (93) and " Compounds I and II " (135). More recently Davoli (92) has isolated a crude antibiotic from *A. ustus* culture filtrates, insufficiently characterized to make it possible to compare it with other antibiotics produced by this species.

Aspergillus flavipes group

Wilkins and Harris (258) first recorded that *A. flavipes* produced a substance selectively toxic to Gram-positive bacteria. White (243) and Foster and Karow (106) both indicated that this was a penicillin.

Aspergillus versicolor group

Production of antibacterial substances by *A. janus* has been recorded by Gill-Carey (119), by *A. versicolor* and *A. sydowi* by Furtado (113). In no case has the substance been isolated in pure form, but it seems possible that a penicillin is involved. *A. humicola* produces a highly antifungal antibiotic, humicolin (91).

Aspergillus terreus group

Marked antibacterial activity is shown by all members of this group. At least four antibiotics have been isolated. Timonin reported production of citrinin (228) from an organism which he attributed to *A. candidus;* subsequently Thom and Raper (227) showed that this was in fact *A. niveus,* a member of the *A. terreus* group. Citrinin has also been obtained from *A. terreus* (153); this species has been shown to produce two other antibacterial substances—terreic acid (102) and the chlorine-containing substance geodin. Geodin was first isolated in 1936 (76, 208), but its antibacterial properties were not realized until 1947 (166).

Aspergillus candidus group

Strains of *Aspergillus candidus* have been examined by several workers (113, 258, 261) with negative results. More recently Stansly and Ananenko (224), using a freshly isolated strain, identified by K. B. Raper, have isolated a substance which they have named " candidulin ", active against acid-fast bacteria.

Aspergillus niger group

Production of an antibacterial substance has been recorded for the following species: *A. cinnamomeus* (258), *A. fonsecaeus* (119), *A. fumaricus* (258), *A. luchuensis* (258, 261), *A. niger* (106, 156, 261), *A. phoenicis* (119), *A. schiemanni* (258, 261). In one case with *A. niger* (106) the activity has been attributed to penicillin; in most cases, however, the antibacterial spectrum was wider than would be expected for penicillin, and other substances must be involved.

Aspergillus wentii group

Antibacterial activity is exhibited by several members of this group—*A. alliaceus* (119), *A. panamensis* (119) and *A. wentii* (119, 261). The mildly antibacterial kojic acid has been isolated from *A. alliaceus* (120) and *A. wentii* (120, 272).

Aspergillus tamarii group

In this group, as in the *wentii* group, antibiotic activity seems attributable to kojic acid in most cases. It has been obtained from *A. lutescens* (102, 119), *A. tamarii* (123) and *A. luteo-virescens* (= *A. tamarii*) (177).

Aspergillus flavus-oryzae group

Here, too, kojic acid production is frequently responsible for all or part of the antibacterial activity developing in cultures of species of this group. It has been isolated from *A. effusus* (141), *A. flavus* (169), *A. oryzae* (272) and *A. parasiticus* (28). However, penicillin-like substances, sometimes known as "flavicin" and "flavicidin", have been reported from *A. flavus* (65, 66, 164, 165, 232) and from *A. parasiticus* (parasiticin) (81). Two other antibiotics, aspergillic acid and hydroxyaspergillic acid, have also been obtained from *A. flavus* (144, 244).

Aspergillus ochraceus group

In this group the following species have been found to produce antibiotics: *A. melleus* (119), *A. ochraceus* (113, 258, 261), *A. quercinus* (119) and *A. sulphureus* (119). From *A. melleus*, Burton (64) has isolated penicillic acid, the weakly-antibacterial substance mellein, and a third unnamed antibacterial acid ($C_{17}H_{22}O_8$). It appears that the activity of all these species is largely based on the production of mellein and penicillic acid, since mellein has also been isolated from *A. ochraceus* (181), and penicillic acid from *A. ochraceus* (146), *A. quercinus* (120) and *A. sulphureus* (120).

Botrytis. The production of antibacterial culture filtrates by *Botrytis cinerea* has been recorded (82, 258), but no antibiotic has actually been extracted.

Cephalosporium. Robbins et al. (216) have tested a strain of *Cephalosporium acremonium* and found no activity, but more recently Brotzu (62) has reported production of culture filtrates toxic to Gram + and Gram – bacteria.

Cladosporium. The interesting mould *Cladosporium cellare* has been shown to produce an antibacterial substance which has been obtained in crystalline form but which is not as yet named or adequately characterized (113*a*).

Epidermophyton. E. inguinale (217) and *E. floccosum* (191) have been reported to produce antibacterial substances. No attempt at isolation appears to have been made; it was suggested that the antibiotic produced by *E. floccosum* is a penicillin.

Fusarium. This large genus shows considerable promise for the production of antibiotics; of 19 species examined, 12 have been found active. It is therefore surprising to find that, of the 16 sections into which Wollenweber and Reinking (266) divide the genus, no less than nine appear to have been completely neglected, viz., *Eupionnotes, Macroconia, Spicarioides, Submicrocera, Pseudomicrocera, Arachnites, Sporotrichiella, Arthrosporiella* and *Ventricosum.*

From many of the species tested (considered below under their respective sections) antibiotics have been isolated in pure or almost pure form. Apart from the well-characterised quinone pigments, javanicin and oxyjavanicin, and the peptide lycomarasmin, there are a number of antibacterials with very similar properties. In this latter group a considerable number of allegedly distinct substances have been described. Cook et al. have described substances under the names lateritiin I, lateritiin II, avenacein, fructigenin and sambucinin (80). Plattner and Nager (202) have described three substances, enniatin A, enniatin B and enniatin C, of which only the first two have actually been isolated in pure form, though the evidence for the existence of enniatin C, based on the finding of a distinct product of hydrolysis, is convincing. These workers have shown that the three enniatins usually occur as mixtures, from which it is difficult to separate the constituents, and have suggested that the substances described by Cook et al. are in fact mixtures of two or three of the enniatins. Since the point is not finally settled (79), all the named antibiotics produced by Fusaria are discussed separately below.

Section Avenaceum. *F. avenaceum* has been shown (80, 82) to produce the antibiotics avenacein and enniatins A and B (202).

Section Gibbosum. *F. scirpi* (202) produces enniatins A, B and C.

Section Discolor. *F. culmorum* produces an antibiotic more toxic to *Mycobacterium phlei* than to other organisms, but this has not been isolated (11, 80, 82). *F. sambucinum* has yielded the antibiotics sambucinin (11, 80, 82) and enniatin A, B and C (202).

Section Lateritium. From *F. fructigenum* the antibiotic fructigenin has been obtained, and from *F. lateritium* the antibiotics lateritiin I and lateritiin II (11, 80, 82). It has been suggested that lateritiin I is identical with enniatin A but that it is frequently contaminated with enniatins B and C and possibly another substance (202).

Section Liseola. *Fusarium moniliforme* is the conidial stage of *Gibberella fujikuroi* from which the antibiotic fusaric acid has been isolated (275).

Section Elegans. From various strains of *F. oxysporum* the antibiotics enniatin A, enniatin B and enniatin C have been isolated (116, 199, 202, 203). It is worth recording here that the organism from which enniatin A was first obtained was initially regarded (116) as a form of *F. orthoceras,* for which the new varietal name *enniatinum* was proposed; subsequently this strain was found to be *F. oxysporum* (202). From the closely related *F. hyperoxysporum,* Texera (225) has described the isolation in crude form of two antibiotics which, like those previously described in this section, were more toxic to Gram + and acid-fast organisms than to Gram – organisms; he also showed them to be mildly antifungal; they, too, may well be mixtures of enniatins.

From *F. lycopersici* (= *F. bulbigenum* var. *lycopersici*) Plattner and Clauson-Kaas (197, 198) isolated a peptide which they named "lycomarasmin". This material is toxic to higher plants, causing wilting (114, 115), and is also antibacterial (270) and antifungal (46). From other strains of *F. bulbigenum* the production of an antibacterial substance has been reported and also from *F. apii* (*F. orthoceras* v. *apii*) (225) and *F. dianthi* (80).

Section Martiella. From *F. javanicum,* Arnstein et al. (11) isolated two antibacterial and antifungal naphthaquinones named "javanicin" and "oxyjavanicin". *F. caeruleum* is said (56) to produce an antifungal substance but this has not been isolated.

Gliocladium. It is widely stated in the literature that *Gliocladium fimbriatum* is one of the organisms which produce the antibiotic gliotoxin. This is undoubtedly an error, but the circumstances in which this specific name has been used are confusing and it is perhaps worth clarifying the situation here. Gliotoxin was first described by Weindling and Emerson (242) as a "lethal principle" produced by *Trichoderma lignorum* (Tode) Harz (= *T. viride* Pers. ex Fries). Weindling subsequently (240), on the

advice of C. Thom and M. Timonin, reported that he had misidentified the fungus which should have been described as *Gliocladium fimbriatum* Gilman and Abbott. He pointed out that his fungus differed from Gilman and Abbott's description (122); nevertheless, this new identification became generally accepted. Subsequently Brian (42) queried this use of the name *G. fimbriatum;* he showed that strains of *Trichoderma viride* produced gliotoxin, that these appeared to be identical with Weindling's organism and that both differed from the published description of *G. fimbriatum* and from an organism (NCTC. 6599) which purported to be *G. fimbriatum.* Some years later Raper and Thom (210) accepted Brian's point of view regarding Weindling's organism and further pointed out that in their view *Gliocladium fimbriatum* was not a valid species, and they considered that the organism described under that name by Gilman and Abbott was really *Myrothecium verrucaria.* The culture of *G. fimbriatum* in the Baarn collection, which is believed to be Gilman and Abbott's type unchanged, is undoubtedly *M. verrucaria* and vigorously produces (46) the antibiotic glutinosin described by Brian et al. (49, 61). The organism tested by Brian and by Wilkins and Harris (260) as *G. fimbriatum* (NCTC. 6599) is now considered (46) to be *Gliocladium catenulatum* Gilman and Abbott. Thus the name *Gliocladium fimbriatum* in the literature on gliotoxin should be replaced by *Trichoderma viride.*

Gliocladium catenulatum produces antifungal and antibacterial culture filtrates (56, 260), and *G. deliquescens* produces antibacterial culture filtrates (260). In neither case have antibiotics been isolated.

Two coloured compounds, aurantiogliocladin and rubrogliocladin, and one colourless compound, gliorosein, have been isolated from cultures of a soil fungus approximating to *Gliocladium roseum* (54); they are moderately inhibitory to Gram + bacteria; (See under GLIOCLADIUM ANTIBIOTICS on page 395).

Metarrhizium. See *Myrothecium.*

Microsporon. Robbins et al. (216) have reported that *M. canis* produces an antibiotic toxic to Gram + organisms. This may be penicillin-like, as has been suggested in the somewhat similar case in the dermatophytic genus *Epidermophyton.*

Myrothecium. A fungus described as *Metarrhizium glutinosum*

Pope, subsequently shown to be the well-established species *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fries (245), produces a highly antifungal antibiotic of negligible antibacterial activity, glutinosin (49, 61). It was subsequently found (57) that other strains of *Myrothecium verrucaria* and *M. roridum* produce similar antifungal antibiotics, and later (58) this was shown to be true also of *M. jollymannii*. The species *M. inundatum, M. gramineum* and *M. striatisporum* do not produce antifungal antibiotics, though there were indications that *M. inundatum* produces an antibacterial substance. There is reason to suppose (89, 162) that other antibiotics, chemically distinct from glutinosin, are involved.

Oidiodendron. Two related compounds, fuscin and dihydrofuscin, have been isolated by Michael (175) from culture filtrates of *Oidiodendron fuscum.*

Oospora. A colourless substance, sulphochracein, mildly inhibitory to Gram + bacteria, has been isolated (132) from culture filtrates of *Oospora sulphurea-ochracea.*

Paecilomyces. Antibiotic activity in culture filtrates from *Paecilomyces burci* and *P. varioti* has been reported (102, 260), and Burton (63) has shown that *Paecilomyces varioti* (tested under the name *Penicillium divaricatum*) produces a penicillin-like antibiotic.

Penicillium. The large and difficult genus *Penicillium* has received much attention in the search for new antibiotics. A survey of the literature has established that fungi attributed to 213 species have been tested. However, of these, twelve should be referred to other genera (*Paecilomyces, Gliocladium, Scopulariopsis, Aspergillus*), and five refer to species of very doubtful authenticity. This leaves 196 species; this number includes many synonyms and probably corresponds to no more than 123 species in the sense used by Raper and Thom (210). These authors have recognized 137 species; it will be seen that the genus has been very adequately covered. Of the 123 distinct species tested, 74 have been found to produce antibiotics; this corresponds to 105 of the 196 forms given distinct specific names in the literature.

Raper and Thom have grouped the species of this genus into 41 series, and it is convenient to consider each of these in turn. Negative results are not reported in detail, but a list of species of

Penicillium which do not appear to have been tested and of species which have been tested with consistently negative results is given at the end of this section.

Monoverticillata

Penicillium javanicum series

Wilkins and Harris (260) reported that *P. javanicum* produced a substance inhibitory to Gram + bacteria.

Penicillium thomii series

P. thomii (145) produces penicillic acid; a penicillin-like substance has been reported (103) from *P. turbatum.*

Penicillium frequentans series

Several workers (56, 102, 259) have reported antibacterial or antifungal activity for *P. frequentans* (under specific names *frequentans, flavidorsum, glabrum*). A mildly antibacterial substance, frequentic acid, has been described (102); this has subsequently been shown (125*a*), to be identical with the previously described substance, citromycetin. *P. frequentans* also produces an antifungal biotic named "frequentin" (91*a*).

From *P. spinulosum* antibacterial activity has been frequently recorded under the specific names *spinulosum, pfefferianum* and *roseomaculatum* (15, 99, 45, 210). The substances isolated include the antibacterial spinulosin (31) and an antibacterial substance isolated by Kondo and Takahashi (described elsewhere in this review as "*P. spinulosum antibiotic*") (155).

Penicillium lividum series

Pollock (204) has reported the production of citrinin by *P. lividum;* the activity of culture filtrates from the related *P. aurantio-violaceum* (259) may be due to the same substance.

Penicillium implicatum series

P. implicatum also owes its activity to production of citrinin (204, 223, 260).

Penicillium decumbens series

P. cinerascens and *P. phaeo-janthinellum* are considered by Raper and Thom to be synonyms of *P. fellutanum.* From *P. cinerascens,* Bracken and Raistrick (41) have recorded production of the antibiotics gliotoxin and spinulosin, and from *P. phaeo-*

janthinellum, Pollock (204) has recorded the production of citrinin. Citrinin has also been isolated (102) from *P. citreo-sulfuratum* (regarded by Raper and Thom as identical with *P. citreo-viride,* which has been reported earlier (56) to produce antifungal culture filtrates).

Penicillium restrictum series

Both species of the series, *P. restrictum* and *P. fuscum,* have been tested (102, 129, 258) with negative results.

Penicillium adametzi series

From *P. terlikowskii* (43) the antibacterial and antifungal gliotoxin has been obtained. A strain at first identified as *P. obscurum,* from which gliotoxin was isolated (178), was subsequently found by Raper and Thom (210) to be *P. terlikowskii.* From *P. phoeniceum* a pigment, phoenicin, was isolated by Friedheim (112); this was subsequently found by Burton (63) to be antibacterial. Two other species in this series have been reported to be active: Bjorkman (35) has recorded antifungal activity for *P. adametzi,* and antibacterial activity has been recorded (260) for *P. vinaceum.*

Ramigena series

Three species have apparently been examined but none showed activity.

Asymmetrica-Divaricata

Carpenteles series

Burton (63) has recorded production of penicillic acid by *P. baarnense;* the closely related, if not identical, *P. euglaucum* is said to produce a penicillin (102).

Penicillium raistrickii series

No antibiotic appears to have been isolated from a member of this series, but Wilkins and Harris (259) record that *P. raistrickii* is active against both Gram + and Gram – bacteria.

Penicillium lilacinum series

Antibacterial (56, 260) and antifungal (56) activity has been recorded for *P. lilacinum,* but no antibiotic has been isolated.

Penicillium janthinellum series

Wilkins and Harris have reported antibacterial activity for *P.*

janthinellum (259), *P. daleae* (259) and *P. godlewskii* (260). The activity of *P. daleae* may have been due to kojic acid which was isolated from this species by Birkinshaw et al. (28) in 1931. Penicillin has been reported from *P. godlewskii* (24).

Penicillium canescens series

Antibacterial activity has been recorded for *P. canescens* and *P. chrzaszczi* (= *P. jenseni*) (260). The activity of the latter has been shown to be due to citrinin (204). Brian, Hemming and McGowan (59) recorded the production of gliotoxin by a species regarded by them as *P. jenseni*, but subsequently (43) the species concerned was shown to be *P. terlikowskii*, which falls in another series.

Penicillium nigricans series

This series of four species appears to be versatile in the production of antibiotics. *P. nigricans* (under the name *P. janczewskii*) has been shown by Brian, Curtis and Hemming (48) to produce a substance, which they called " curling-factor ", causing abnormal morphogenesis of some fungi, subsequently shown (51, 126) to be the chlorine-containing substance griseofulvin, earlier obtained from *P. griseofulvum* by Oxford, Raistrick and Simonart (187). The closely related *P. melinii*, produces patulin (145), and *P. raciborskii* yields a penicillin (102). From a strain attributed to the *P. nigricans-janczewskii* series, subsequently determined as *P. albidum*, Curtis and Grove (90) isolated a highly antibacterial and antifungal " red pigment ".

Asymmetrica-Velutina

Penicillium citrinum series

All three species in this series have been associated with production of antibiotics. The antibacterial activity of *P. citrinum* (183, 209, 247, 258) can be explained on the basis of production of citrinin, first isolated by Hetherington and Raistrick (134*a*). The antibiotic activity of *P. sartoryi* (260), regarded by Raper and Thom as identical with *P. citrinum*, can probably be similarly explained. The antibacterial activity of a strain of *P. steckii* (258) has been shown to be due to penicillin (102). From *P. corylophilum* (under the name *P. chloroleucon*) production of penicillin has been demonstrated (142). An antibiotic named " corylophilin ", similar in properties to notatin, has been described

(192, 193, 194, 195); there is reason to believe (210) that the mould concerned may have been a strain of *P. notatum*. The *P. obscurum* (= *P. corylophilum*), from which Mull, Townby and Scholz (178) obtained gliotoxin, has been shown (210) to be *P. terlikowskii* (*adametzi* series).

P. chrysogenum series

Penicillin is produced by all species in this series. Notatin, a glucose-oxidase with antibacterial properties in the presence of glucose, has been obtained from *P. notatum* (85).

P. oxalicum series

No antibiotic activity has been recorded for either of the two species (*oxalicum, atramentosum*) in this series.

Penicillium digitatum series

Some antibacterial activity has been recorded for *P. digitatum* (56, 259), but no antibiotic has been isolated.

Penicillium roqueforti series

Antibacterial activity has been recorded for *P. casei* (102) and antifungal and antibacterial activity for *P. roqueforti* (56). From *P. suavolens* (= *P. roqueforti*) Karow, Woodruff and Foster (146) isolated penicillic acid.

Penicillium brevicompactum series

Antibacterial and antifungal activity has been demonstrated for *P. brevicompactum* and *P. stoloniferum* under a variety of specific names (*brevicompactum, griseobrunneum, hagemi, patris-mei, scabrum, stoloniferum, szaferi, bialowienzense*), and in every case mycophenolic acid has been isolated (63, 77, 102, 104, 259, 260). This has been shown to possess antibacterial and antifungal properties (1, 25, 121). It remains possible that some of the other acids isolated from moulds of this series by Raistrick and his colleagues (77, 78) may be responsible for some of the activity of culture filtrates. A strain of *P. paxilli*, the remaining member of the series, has been found to produce antifungal and antibacterial culture filtrates (58), and again mycophenolic acid was isolated (89). Thus mycophenolic acid and some related acids are completely characteristic of the *brevicompactum* series and responsible for their antibiotic activity.

Asymmetrica-Lanata

Penicillium camemberti series

P. caseicolum is active against Gram+ bacteria, but no antibiotic has been isolated (259).

Penicillium commune series

Antibacterial activity has been reported for *P. lanosum* (259), *P. lanosoviride* (260), *P. lanoso-coeruleum* (259) and *P. aurantio-candidum* (and the identical *P. aurantio-albidum*) (102). In the case of one strain of *P. lanosum* the antibiotic has been identified as penicillin (102).

Asymmetrica-Funiculosa

Penicillium terrestre series

The antibacterial activity of *P. terrestre,* first noted by Wilkins and Harris (259, 260), is probably due to production of patulin. According to Raper and Thom (210), the mould from which Atkinson (12, 13, 15) obtained an antibiotic " penicidin ", later found to be identical with patulin, approximated to *P. terrestre.* Patulin has also been obtained (102) from *P. equinum,* a form considered by Raper and Thom to be the same as *P. terrestre.* Antibacterial activity has also been reported for *P. solitum* (102) and for *P. resticulosum* (33); notatin has been obtained from the latter (85).

Penicillium pallidum series

Chastukhin and Nikolaevskaya (74) have reported the production of penicillin by *P. namylovskii.*

Asymmetrica-Fasciculata

Penicillium gladioli series

Wilkins and Harris (259, 260) first reported antibacterial activity by *P. gladioli.* Brian et al. (47, 50) subsequently isolated an antifungal and antibacterial substance, gladiolic acid. A number of strains producing gladiolic acid approximate closely to *P. corymbiferum* (46) and produce no sclerotia; Thom (226) described the close similarity between one strain of *P. gladioli* (5034.65) and *P. corymbiferum.*

Penicillium ochraceum series

Antibacterial activity has been reported for *P. ochraceum* (259)

and for *P. carneo-lutescens* (102). From the latter a highly antibacterial substance named "carneo-lutescin" was isolated (102) in crude form.

Penicillium viridicatum series

Occasional strains of *P. viridicatum* have been found to be antibacterial (102, 259), and from one of these strains Burton (63) has obtained mycophenolic acid. This is the only occasion on which mycophenolic acid has been isolated from a species outside the *P. brevicompactum* series.

Penicillium cyclopium series

All four species in this series produce antibacterial culture filtrates; the activity can in most cases be attributed to one or more of three acids—penicillic acid, puberulic acid and puberulonic acid. All three have been isolated from culture filtrates, accounting for their antibacterial activity, of *P. cyclopium* (30, 32, 188, 189, 258) and *P. puberulum* (3, 32, 188, 189). Campbell et al. (68) have also recorded the isolation of a nitrogenous antibiotic ($C_{17}H_{12}N_2O_2$) from *P. puberulum* mycelium. *P. martensii* (tested as *P. janthogenum*) has been found active (260), probably attributable to puberulic and puberulonic acids, earlier isolated from this species (189). These same two acids have also been isolated (32) from *P. aurantiovirens* and probably account for the activity of culture filtrates of this species (tested under the name *P. brunneo-violaceum*) (247).

Penicillium expansum series

Penicillium expansum has frequently been observed to be antagonistic to bacteria and fungi (9, 160, 258, 259, 260), and the antibiotic patulin has been isolated by several workers (9, 99, 153, 179). *P. crustosum* has been shown to produce antibacterial culture filtrates (259). Miller and Rekate (176) demonstrated the presence of a substance toxic to acid-fast bacteria in the mycelium of a species of *Penicillium* later identified by K. B. Raper as *P. crustosum*. Riley and Miller (214) isolated the substance and showed that it was linoleic acid; it did not accumulate in the culture medium. The mould of this name from which Yermolieva et al. (276) produced an antibacterial substance ("penicillin-crustosin") was probably (210) *P. notatum*. Possibly the strain of *P. crustosum* from which Lyanda-Geller and Markovich (161) obtained penicillin was the same form.

Penicillium italicum series

One strain of *P. italicum*, of several tested (258, 259, 260), was found to produce an antibacterial substance; no antibiotic has been isolated.

Penicillium urticae series

The two species in this series are very versatile biochemically, and quite a number of their metabolic products are antibiotic. Culture filtrates of both *P. urticae* (= *P. flexuosum*, *P. patulum*) (26, 29, 259, 260) and *P. griseofulvum* (51, 102) have been found to be antifungal and antibacterial, and the activity can be accounted for by the following substances—patulin from *P. urticae* (*patulum*) (26, 29), griseofulvin from *P. urticae* (*patulum*) and *P. griseofulvum* (51, 187), gentisyl alcohol from *P. urticae* (*patulum*) (26), mycelianamide from *P. griseofulvum* (185, 187), a penicillin from *P. griseofulvum* (142) and 6-methyl-salicylic acid from *P. urticae* (*flexuosum*) and *P. griseofulvum* (5, 186, 206).

Penicillium granulatum series

Raper and Thom recognise two species in this series, *P. granulatum* and *P. corymbiferum*. From *P. divergens* (= *P. granulatum*), Barta and Mecir (18) have isolated the antibiotics patulin and gentisyl alcohol. Many strains of *P. corymbiferum* have been examined with negative results (258, 259, 260), but a strain of *P. hirsutum* (= *P. corymbiferum*) was found to produce an antibacterial substance (259); no attempt at isolation has been recorded. Gladiolic acid has been isolated from strains which approximate more closely to *P. corymbiferum* than to *P. gladioli* (see under *P. gladioli* series).

Penicillium claviforme series

Patulin and 6-methyl-salicylic acid have also been isolated from *P. claviforme* (71, 72, 124).

Biverticillata-Symmetrica

Penicillium luteum series

A penicillin has been recorded from *P. avellaneum* (103, 260), and an antibacterial substance, not yet isolated, has been reported for *P. wortmanni* (102). Waksman and Horning (234) record antibacterial activity for what they termed the *P. luteum-purpurogenum* group. No activity appears to have been detected in culture

filtrates from *P. stipitatum*, but a metabolic product of this mould, stipitatic acid, has been found by Rennerfelt (213) to be antifungal.

Penicillium duclauxi series.

Production of a substance toxic to Gram + bacteria by *P. duclauxi* has been recorded (259), but no record of its extraction has been found.

Penicillium funiculosum series

Antibacterial activity has been demonstrated (258) in culture filtrates from *P. funiculosum*, and Shope (22) states that this species produces a substance which inactivates swine influenza virus.

Penicillium purpurogenum series

Phoenicin has been reported (63, 88) from *P. rubrum*. Waksman and Horning (234) record antibacterial activity for strains of the "*P. luteum-purpurogenum* group".

Penicillium rugulosum series

Activity against Gram + bacteria has been recorded for *P. chrysitis* (260) and *P. crateriforme*, both regarded by Raper and Thom as synonyms of *P. rugulosum*, and in *P. crateriforme* the activity has been attributed to penicillin (142). From *Penicillium tardum* the antibiotic tardin has been isolated (36). The activity reported for *P. elongatum* (102), which is a synonym of *P. tardum*, may be due to the same substance.

Penicillium herquei series

Antibacterial activity has been reported for all three members of this series—*P. herquei* (259), *P. olsoni* (259) and *P. novaezeelandiae* (102). From *P. herquei* a mildly antibacterial substance named "herquein" has been isolated (63); from *P. novaezeelandiae* patulin has been isolated (63).

In Table II a summary of the results obtained with *Penicillium* spp. is given; the species recognized by Raper and Thom are divided into three groups: active; inactive in such tests as have been applied; and those for which no record of test can be traced.

Stachybotrys. Brian and Hemming (56) have reported that culture filtrates of several strains of *S. atra* show antifungal and

antibacterial activity. Curtis (89) has succeeded in preparing crude concentrates showing activity but has not isolated any pure materials. Some confirmation of the activity of *S. atra* has been provided by Robbins (see 246).

Torula. Uroma and Virtanen (230) have claimed that *Torula utilis* produces an antibacterial substance; no attempt at isolation is reported.

Trichoderma. In a series of researches Weindling (238, 239) studied the antagonism between *Trichoderma viride* (= *T. lignorum*) and other fungi, particularly *Rhizoctonia solani.* He was able to show that the antagonistic powers of *Trichoderma* were associated with the power to secrete a lethal principle toxic to other fungi. This was subsequently isolated and named "gliotoxin" (55, 240, 241, 242), and was found to be powerfully antibacterial and antifungal. Other strains of *T. viride* produce an extremely antifungal substance, viridin, without any effect on bacteria (52, 60). It seems probable that most strains produce a mixture of the two antibiotics, the proportions varying from strain to strain. Some strains of *T. viride* produce activity suggesting that other antibiotics are also produced (56).

Trichophyton. Several species of *Trichophyton,* including *T. mentagrophytes, T. purpureum, T. tonsurans* and *T. violaceum,* have been shown (191, 216) to produce antibiotics inhibitory to Gram + bacteria, and it has been suggested that production of a penicillin is involved (191).

Trichothecium. T. roseum has long been known to be antagonistic to other fungi, and Brian and Hemming (56) confirmed that it produced an antifungal substance when grown on liquid media. Freeman and Morrison (108, 109, 110) isolated an antifungal (but not antibacterial) substance, which they named "trichothecin", and subsequently a substance—rosein II—which is antibacterial (111).

From this survey of antibiotic production by Fungi Imperfecti, it emerges that about 66 distinct antibiotics have been isolated in pure form or as substantially pure concentrates. Although some of these are produced by many different species, even different genera (e.g., penicillin, penicillic acid, gliotoxin, citrinin, patulin),

TABLE II

ANTIBIOTIC ACTIVITY OF SPECIES OF PENICILLIUM

Series	Active	Inactive	Not tested
javanicum	javanicum	brefeldianum	parvum, ehrlichii, levitum
thomii	thomii, turbatum	sclerotiorum	lapidosum, pusillum
frequentans	frequentans, spinulosum		purpurrescens
lividum	lividum, aurantio-violaceum	trzebinskii	
implicatum	implicatum	multicolor, sublateritium	
decumbens	fellutanum, citreo-viride	chermesinum, roseo-purpureum, decumbens	
restrictum		restrictum, fuscum	
adametzi	adametzi, terlikowskii, phoeniceum, vinaceum		
Ramigena		charlesii, waksmani, velutinum	capsulatum, cyaneum
Carpenteles	baarnense		asperum, egyptiacum
raistrickii	raistrickii	pulvillorum, soppi	rolfsii
lilacinum	lilacinum	humuli	Spicaria violacea
janthinellum	daleae, godlewskii, janthinellum	piscarium, miczynskii	simplicissimum, ochro-chloron
canescens	canescens, jenseni		nalgiovensis
nigricans	nigricans, albidum, raciborskii, melinii	kapuscinskii	
citrinum	citrinum, corylophilum, steckii		
chrysogenum	chrysogenum, meleagrinum, notatum, cyanofulvum		
oxalicum		oxalicum, atramentosum	
digitatum	digitatum		
roqueforti	roqueforti, casei		
brevicompactum	brevicompactum, stoloniferum, paxilli		

TABLE II (*continued*)

Series	Active	Inactive	Not tested
camemberti	caseicolum	camemberti	
commune	lanosum, lanoso-viride, lanoso-coeruleum, aurantio-candidum	biforme, commune, lanoso-griseum	
terrestre	terrestre, solitum, resticulosum	psittacinum	
pallidum	namylovskii	pallidum, putterilli	
gladioli	gladioli		
ochraceum	ochraceum, carneo-lutescens		
viridicatum	viridicatum, palitans	olivino-viride	
cyclopium	cyclopium, puberulum, martensii, aurantio-virens		
expansum	expansum, crustosum		
italicum	italicum		
urticae	urticae, griseofulvum		
granulatum	granulatum, corymbiferum		
claviforme	claviforme	clavigerum	
luteum	avellaneum, wortmanni	duponti, stipitatum, spiculisporum, bacillosporum, luteum	vermiculatum, helicum, rotundum, striatum
duclauxi	duclauxi		
funiculosum		funiculosum, islandicum, varians	verruculosum, piceum
purpurogenum	rubrum	purpurogenum	aculeatum, variabile
rugulosum	rugulosum, tardum		diversum
herquei	herquei, olsoni, novae-zeelandiae		
Polyverticillata			albicans

others are known only from single species (e.g., viridin, aspergillic acid, glutinosin, trichothecin, mycelianamide). In fact there is a distinct tendency for each genus, or group of related species within a genus, to have its characteristic antibiotics. This can be seen even in genera, such as *Aspergillus* or *Penicillium*, which consist of large numbers of species, many of which are closely related to each other. Thus, for instance, though kojic acid is produced outside the genus, it is particularly characteristic of the species in the related *wentii, tamarii* and *flavus-oryzae* groups of the genus *Aspergillus;* mycophenolic acid is characteristic of the *Penicillium brevicompactum* series; in the sub-section Fasciculata of the genus *Penicillium* three groups of species can be discerned on the basis of antibiotic production: (*a*) gladiolic acid producers—*gladioli* series and perhaps *P. corymbiferum;* (*b*) patulin—gentisyl alcohol —hydroxy-methyl-benzoic acid—griseofulvin producers in the *expansum, urticae, claviforme* and *granulatum* series; and (*c*) penicillic acid—puberulic acid—puberulonic acid producers in the *cyclopium* series.

Since this group specificity exists, though masked by production of certain antibiotics by a wide range of species and genera, the prospects of finding new antibiotics from other genera in the Fungi Imperfecti can be assessed. Records indicate that out of the 1234 genera known, only 47 have been investigated. Moreover, as in the Ascomycetes, the ground has been covered very unevenly; the orders Sphaeropsidales and Melanconiales, which have been almost completely neglected, should repay investigation. At the same time the prospects of finding many new antibiotics from genera in the Moniliales are quite promising.

TABLE III

BASIDIOMYCETES

Order	Total genera	Genera tested	Genera active	Total species	Species tested	Species active
Tremellales	55	14	5	500	52	12
Ustilaginales	35	1	1	700	2(?)	2(?)
Agaricales	172	106	71	6,900	1,579	549
Phallales	21	4	1	65	5	1
Lycoperdales	21	5	1	160	18	3
Sclerodermatales	21	5	1	110	7	1
Nidulariales	7	2	2	55	2	2

BASIDIOMYCETES

No species in the orders Uredinales, Exobasidiales and Hymenogatrales has been tested; this is inevitable in the case of the Uredinales, since it has not yet been found possible to grow them in artificial culture, and the remaining two orders are relatively small, accounting for only 240 species in 40 genera. The distribution of active genera and species in the remaining orders is shown in Table III.

Tremellales

The species which have been found active are *Auricularia auricula-judae, Dacromyces deliquescens, Helicobasidium purpureum* (= *Rhizoctonia crocorum*), *Hirneola* sp., *Inocybe descissa, I. godeyi, I. hirsuta, I. incarnata, I. murrayana, I. obscura, I. pyriodora* and *Tremellodon gelatinosum* (133, 216, 255, 257, 262). None of the antibiotics concerned has been isolated.

Ustilaginales

Haskins (130) has recently described two antibiotics produced by *Ustilago zeae*. These are ustizeain A, which is highly antibacterial, and ustizeain B, which is antifungal.

Agaricales

In this very large order there are at least 6,900 species in 172 genera, but, as will be seen from Table III, a considerable proportion of these have been tested (the figure given for genera tested is somewhat too large, as several synonyms have undoubtedly been included). For convenience each of the six families of the order is considered separately.

Thelephoraceae

In this family 53 species from six genera have been found active. These include species in the genera *Coniophora, Corticium, Craterellus, Hymenochaete, Peniophora* and *Stereum* (133, 216, 249–257, 262). From species of the latter genus, a number of antibiotics have been isolated. From cultures of *Stereum hirsutum*, Heatley, Jennings and Florey (131) have obtained a highly antibacterial substance, hirsutic acid, more effective against Gram + than against Gram – organisms. From cultures of *Stereum*

rameale a weakly antibacterial substance known as "ramealin" has been obtained (102).

Clavariaceae

Ten species from three genera have been found active. These include seven species of *Clavaria* (168, 249, 262), *Lachnocladium* sp. (168), *Sparassis crispa,* from which both antibacterial (133, 216) and antifungal (249) activity have been recorded, and *Sparassis ramosa.* The activity of the latter fungus is of some historical interest. Falck (101) noted that cultures remained remarkably free from contamination, and in 1923 isolated in pure form an antifungal substance which he named "sparassol", which is therefore one of the earliest antibiotics to be isolated in pure form.

Hydnaceae

In this family 19 species from six genera have been found active. These include *Acia stenodon* (262), eleven species of *Hydnum* (133, 216, 250, 254, 255, 262) four species of *Irpex* (133, 216, 254, 255, 262), *Odontia bicolor* (257), *Phlebia strigozonata* (255) and *Radulum orbiculare* (216, 255). From *Irpex destruens* (102) a strongly antibacterial yellow oil, named "destruin", has been isolated.

Polyporaceae

A great number of species in this family have been tested, and not less than 198 species have been found active, from the genera *Daedalea, Fistulina, Fomes, Ganoderma, Lenzites, Merulius, Polyporus, Polystictus, Poria, Ptychogaster, Trametes* and *Ungulina* (249, 250, 252–257, 262). Several antibiotics have been isolated in more or less pure form. From *Ganoderma oregonense,* a crystalline substance highly active against Gram + and acid-fast bacteria has been isolated (102) and named "oregonensin". From *Polyporus biformis* the highly antibacterial biformin and a much less active substance, biforminic acid, have been isolated (219). Bose (39, 40) has described a crude antibacterial substance, obtained from *Polyporus* spp. and *Polystictus sanguineus,* under the name "polyporin". Meyer (173, 174) has similarly prepared crude extracts of *Polyporus cinnabarinus* showing marked activity against Gram + organisms. Polystictin (102) is a highly specifically antibacterial substance isolated in crude form

from *Polystictus versicolor.* From *Poria corticola* and *Poria tenuis,* Kavanagh, Hervey and Robbins (150) have isolated, apparently in substantially pure form, two highly antibacterial and antifungal substances named " nemotin " and " nemotinic acid ". The antibacterial substance occurring in sporophores of *Ungulina betulina,* at first known as " ungulinic acid ", has been shown (158) to be polyporenic acid A, one of the three substances earlier isolated by Cross et al. (87) from the same fungus (in this case reported under the name *Polyporus betulinus*).

Boletaceae

Eleven species of *Boletus* have been found active, but no antibiotic has been isolated (249, 250, 252, 255, 262).

Agaricaceae

No less than 258 active species have been found in the following 43 genera: *Agrocybe, Alnicola, Amanita, Armillaria, Bolbitius, Cantharellus, Claudopus, Clitocybe, Clitopilus, Collybia, Coprinus, Cortinarius, Crepidotus, Dermocybe, Drosophila, Flammula, Galera, Gomphidius, Hebeloma, Hygrophorus, Hypholoma, Inoloma, Lactarius, Lentinus, Lepiota, Leptonia, Marasmius, Mycena, Naucoria, Omphalia, Panaeolus, Panus, Paxillus, Pholiota, Pleurotus, Pluteus, Psalliota, Psilocybe, Rhodopaxillus, Russula, Stropharia, Tricholoma* and *Trogia* (133, 168, 216, 249, 250, 252–257, 262). Sixteen apparently distinct antibiotics have been isolated. A generally antibacterial and antifungal substance, named " agrocybin ", has been isolated from *Agrocybe dura* (151). Clitocybine B has been isolated in crude form by Hollande (136–139) from *Clitocybe giganteus;* it is highly active against acid-fast and Gram + bacteria. A substance, named " nebularine " (159) and isolated from *Clitocybe nebularis,* also showed high activity against acid-fast organisms and may be related to or identical with clitocybine B. From *Clitocybe illudens* two antibiotics have been isolated and named " illudin M " and " illudin S " (4*a*); these are both active against Gram + and acid-fast bacteria. From *Coprinus picaceus,* a yellow oil, named " picacic acid ", has been isolated (102); it is mildly antibacterial. 4-methoxy-2: 5-toluquinone, an antifungal and antibacterial substance, related chemically to the antibiotics spinulosin and fumigatin, is produced by *Coprinus similis* and *Lentinus degener* (4). A crude antibiotic,

inolomin, showing rather specific antibacterial properties, has been isolated by Fragner (107) from *Inoloma traganum*. A violet pigment, lactaroviolin, isolated from the sporophores of *Lactarius deliciosus*, is moderately active in vitro against *Mycobacterium tuberculosis* (147, 263, 264).

Five antibiotics appear to have been isolated from species of *Marasmius*. Kavanagh, Hervey and Robbins (149) have isolated a substance showing marked antibacterial activity and slight antifungal activity from *Marasmius conigenus*—marasmic acid. Evidence has been produced for the production of four antibiotics by *Marasmius ramealis* and *M. graminum*, two from each species (21, 22, 170). At least one of these (from *M. graminum*) has been obtained in substantially pure form. A highly antibacterial substance, named "pleurotin", has been isolated in pure form from cultures of *Pleurotus griseus* (218). Nudic acids A and B are two antibacterial substances isolated in pure form from *Tricholoma nudum* (102). A crude antibiotic extract has been prepared from *Tricholoma georgii* (221).

It is worthy of note, in passing, that of these 16 antibiotics produced by species in the Agaricaceae, only six can reasonably be supposed to have been pure substances.

Phallales

A species of *Ithyphallus* shows some antibacterial activity (216).

Lycoperdales

Three species of *Lycoperdon* have shown some antibacterial activity—*L. gemmatum* (216), *L. giganteum* (262) and *L. pyriforme* (216, 257).

Sclerodermatales

Tulostoma subfuscum shows antibacterial activity (168).

Nidulariales

Crucibulum vulgare (251) and *Cyathus stridus* (253, 257) show antibacterial activity.

The Basidiomycetes are quite clearly a potentially fruitful source of antibiotics. Though the Agaricales have been intensively in-

vestigated, the remaining orders have been relatively neglected. Active investigation of the nature of the antibiotics produced has only recently commenced, so that, although 586 active species are known, only 27 antibiotics have been described. Of these, only about half seem to have been isolated in a state of purity sufficient to make it possible to characterise them chemically and physically, and the structures of only two (sparassol and 4-methoxy-2: 5-toluquinone) are known. At present it appears that such Basidiomycete antibiotics as have been obtained in a state of substantial purity are distinct from those reported from other groups of fungi.

FUNGAL ANTIBIOTICS

In this section each of the antibiotics already mentioned is briefly characterised in alphabetical order. Details of chemistry are not discussed, but the more important physical data, simple colour reactions, examples of biological activity and indications of stability are given wherever the data are available. The references in general relate to the physical, chemical and biological characteristics of the isolated antibiotic. References to the conditions under which they are produced may best be found by referring back under the appropriate fungi. Chemical structures (Table 4), where these have been proposed, are grouped together in order that chemical relationships between antibiotics may easily be appreciated. The chemistry of antibiotics has recently been reviewed by Wintersteiner and Dutcher (265).

In each case the fungi known to produce a given antibiotic are listed; those antibiotics which have been isolated from culture filtrates are designated (C), those isolated from fungus mycelium are designated (M), and those isolated from sporophores (Basidiomycete and Ascomycete) are designated (S).

AGROCYBIN. Has been obtained as colourless crystals blackening at about 145°. Soluble in many organic solvents but insoluble in hexane; slightly soluble in water. Inhibits growth of Gram +, Gram –, and acid-fast bacteria, and of fungi. Aqueous solutions are thermostable at pH 6.5 but not at pH 8.5 or higher. Produced by *Agrocybe dura* (C) (151).

ALTERNARIC ACID. Optically inactive, colourless crystals, m.p. 134°. Orange colour with ferric chloride. Highly soluble in chloroform; less soluble in ethanol, carbon tetrachloride and ben-

zene. Sparingly soluble in water but readily soluble in sodium carbonate solution. Not apreciably antibacterial. Its antifungal activity is highly specific; germination of spores of *Absidia glauca, Myrothecium verrucaria* and *Stachybotrys atra* is inhibited in the range 0.1–1.0 μg./ml.; germination of spores of *Botrytis alli* and other fungi is not inhibited appreciably at 200 μg./ml., though elongation of the germ tubes ceases or is reduced in rate soon after they are formed. It is highly phytotoxic. Aqueous solutions are thermostable at pH 3–7.6. Produced by *Alternaria solani* (C) in yields of 100 mg./l. (53).

ASPERGILLIC ACID ($C_{12}H_{20}N_2O_2$). Pale yellow elongated rods, m.p. 93°, $[\alpha]_D^{24} = +12°$. Gives a deep red colour with ferric chloride in methanol solution. The cupric salt (m.p. 198°), which crystallises from ethanol, has a characteristic grass-green colour. Soluble in organic solvents except petrol ether; sparingly soluble in water. Structure XXII. Toxic to Gram + and Gram – bacteria, including plant pathogens, being bacteriostatic in broth in the range 2–50 μg./ml. Growth of fungi is inhibited in agar in the range 50–200 μg./ml. Iron interferes with, and bismuth enhances, antibiotic activity. Produced by *Aspergillus flavus* (C) in yields of 800–1000 mg./l. (19, 94–97, 144, 229, 244, 265, 267).

ASPERGILLUS CAESPITOSUS ANTIBIOTIC. Has been isolated in crude form only. Soluble in ethanol; slightly soluble in benzene and ether. Insoluble in water but soluble in sodium bicarbonate solution, giving a purple solution. It shows weak activity against *Staph. aureus*. Isolated from *Aspergillus caespitosus* (C) (102).

ASPERGILLUS USTUS ANTIBIOTICS. A number of antibiotics have been isolated in relatively pure form from culture filtrates; all contain chlorine, and all are active against Mycobacteria.

Hogeboom and Craig (135) obtained three compounds with the following characteristics: Compound I, m.p. 185–187°, $C_{21}H_{17}Cl_3O_6$ (?), heavy square plates; soluble in organic solvents, almost insoluble in water, inhibiting *Mycobacterium ranae* at 3.3 μg./ml.; Compound II, m.p. 214–216°, $C_{21}H_{18}Cl_2O_6$ (?), colourless needles; soluble in organic solvents, almost insoluble in water, inhibiting *Mycobacterium ranae* at 10 μg./ml.; a third compound was also isolated but not described in detail. Doering et al. (93) have isolated, from mycelium and culture filtrates, four substances:

ustin, m.p. 184–186°, $C_{19}H_{15}Cl_3O_5$; aun$_1$, m.p. 155–156°; aun$_2$, m.p. (decomp.) about 270°; aun$_3$, m.p. 221–225°. Ustin, aun$_1$ and aun$_2$ have similar antibacterial activity, inhibiting growth of Gram + and acid-fast organisms in simple media at 2 μg./ml. The activity is reduced in complex media. Ustin is probably identical with Hogeboom and Craig's Compound I.

These substances probably account for the activity of culture filtrates and crude extracts noted by other workers (157, 92).

AUN$_1$, AUN$_2$, AUN$_3$. See ASPERGILLUS USTUS ANTIBIOTICS

AVENACEIN ($C_{25}H_{44}O_7N_2$). Optically active, colourless, m.p. 139°. Soluble in organic solvents, including petroleum ether; sparingly soluble in water. Bacteriostatic in broth to *Mycobacterium phlei* at 6 μg./ml., to *Staph. aureus* at 100 μg./ml. Highly unstable in alkaline solution but heat-stable in neutral and acid solution. Produced by *Fusarium avenaceum* (C) (80).

BIFORMIN. A neutral substance obtained as yet only in solution, since, on evaporation of solvent, decomposition takes place. No colour with ferric chloride. Soluble in water and many organic solvents. Highly bacteriostatic in broth, concentrations limiting growth being: *B. subtilis,* 0.04 μg./ml.; *Bact. coli,* 1.6 μg./ml.; *Myc. phlei,* 0.6 μg./ml.; *Staph. aureus,* 0.3 μg./ml. It is also antifungal. Dilute aqueous solutions are thermostable in the range pH 3–8. It is produced by *Polyporus biformis* (C), in yields of the order of 80 mg./l. (219).

BIFORMINIC ACID. Has been obtained only in solution because, like biformin, it decomposes on evaporation of the solvent. A preparation of low purity gave a brown colour with ferric chloride, probably not due to biforminic acid. Soluble in water, chloroform, ether and ethanol. It is antibacterial, being bacteriostatic at the following levels: *B. subtilis,* 0.2 μg./ml.; *B. coli,* 3–4 μg./ml.; *Myc. phlei,* 3–4 μg./ml.; *Staph. aureus,* 0.7 μg./ml. It is produced by *Polyporus biformis,* along with biformin, but in much smaller quantity (219).

CANDIDULIN ($C_{11}H_{15}NO_3$). Long white glistening needles, m.p. 88–89°, $[\alpha]_D^{24} = +15°$. No colour with ferric chloride. Soluble in many organic solvents and in water; crystallises from hexane. It is antibacterial, acid-fast organisms being particularly

sensitive, and antifungal. Examples of concentrations needed to prevent growth in broth: *B. subtilis,* 10 μg./ml.; *Myc. ranae,* 2.5 μg./ml.; *Staph. aureus,* 320 μg./ml.; *Aspergillus fumigatus,* 80 μg./ml.; *Penicillium puberulum,* 40 μg./ml. It is produced by a strain of *Aspergillus candidus* (C) in yields of 5 mg./l. (224).

CARNEO-LUTESCIN. Isolated in crude form as an oil containing only C, H and O. It gives no colour with ferric chloride. Soluble in ether, benzene and alcohol; sparingly so in water. It has been found highly active against certain Gram + organisms. Lowest inhibiting concentrations in broth are: *Staph. aureus,* 0.6 μg./ml.; *Str. pyogenes,* 1.2 μg./ml.; *B. anthracis,* 0.15 μg./ml. It is stable in aqueous solution in the range pH 2–6, but at pH 9.6 activity is lost rapidly. It is produced by *Penicillium carneo-lutescens* (C) (102).

CHETOMIN (CHAETOMIN) ($C_{16}H_{17}O_4N_3S_2$ (probable)). White crystalline powder, m.p. 215°, $[\alpha]_D^{22} = +360°$. Soluble in acetone, ethyl acetate, benzene and chloroform; insoluble in water and petroleum ether. Its antibacterial spectrum is similar to that of penicillin, Gram + organisms being particularly sensitive (*Staph. aureus* inhibited in agar at 0.002 μg./ml.). Emulsions in water are thermostable if neutral or acid, but rapidly inactivated if alkaline. Chetomin is produced by *Chaetomium cochlioides, C. spirale* and probably by other species of *Chaetomium* and by *Verticillium cinnabarinum.* In *C. cochlioides* the antibiotic is mostly found in the mycelium, only 5% of the total yield coming from the medium (117, 118, 233).

CITRININ ($C_{13}H_{14}O_5$). Bright yellow needles or plates, m.p. (decomp.) 175°, $[\alpha]_D^{18} = -37.4°$. With ferric chloride it gives a buff precipitate dissolving in excess reagent to give an iodine-brown solution. If a solution is treated with hydrogen peroxide and then made alkaline with sodium hydroxide, a deep red colour develops. It is soluble in chloroform and acetone, less soluble in ethanol or ether; it is practically insoluble in cold water but readily soluble in sodium carbonate solution. The structure originally proposed by Coyne, Raistrick and Robinson (86) was not confirmed by synthesis; more recently Cartwright, Robertson and Whalley (69) have proved structure XVII by synthesis. It is moderately antibacterial; minimum inhibitory concentrations in broth are: *Staph. aureus,* 30–40 μg./ml.; *B. subtilis,* 5 μg./ml.;

B. coli, and *S. typhi*, 500 μg./ml. Aqueous solutions are quite stable in a physiological pH range. It is produced by a number of fungi, including *Penicillium citrinum, P. lividum, P. implicatum, P. chrzaszczi, Aspergillus terreus* and *A. niveus*. From *P. citrinum* yields as high as 4 g./l. (C) can be obtained (134, 183, 188, 204, 207, 209).

CITROMYCETIN ($C_{14}H_{10}O_7 \cdot 2\,H_2O$). Optically inactive, yellow needles, m.p. (decomp.) 283–285°. Gives a deep green colour with ferric chloride. Soluble in chloroform and alcohol, sparingly soluble in water; dissolves in sodium bicarbonate solution with evolution of CO_2. Citromycetin is weakly antibacterial and is identical with a substance described under the name "frequentic acid" (q.v.) (102, 125*a*). Citromycetin is produced (C) by species of the *Penicillium frequentans* series (134).

CLAVACIN
CLAVATIN
CLAVIFORMIN } See PATULIN.

CLITOCYBINE B. Has not been obtained in pure form. The name was first applied to a crude extract of sporophores of *Clitocybe giganteus* (136–139); subsequently other workers (215) obtained a more active material from mycelium grown in artificial culture. The more active ether-soluble fraction was soluble in water, acetone and ether, but not in petroleum ether. It is highly bacteriostatic; the most active preparations prevent growth of a range of Gram +, Gram – and acid-fast organisms at concentrations of 1–4 μg./ml. Aqueous solutions are unstable.

CORYLOPHILIN. See NOTATIN.

DESTRUIN. Has been obtained in partially purified form as a yellow oil containing only C, H and O. Soluble in benzene, ether, ethanol and acetone; sparingly soluble in water. Growth of *Staph. aureus* in broth is inhibited at 100 μg./ml. Aqueous solutions are stable in the range pH 2–7, but at pH 9 activity is slowly lost. Produced by *Irpex destruens* (C) (102).

DIHYDROFUSCIN ($C_{15}H_{18}O_5$). Optically inactive, colourless rhombic crystals, m.p. 206°. Gives a red colour with ferric chloride in ethanol. Moderately soluble in ethanol and ether; almost insoluble in petrol ether or water. Aqueous solutions are readily

oxidised to give the related product fuscin (q.v.). Produced by *Oidiodendron fuscum* and may be isolated from culture filtrates if precautions are taken to prevent oxidation (175).

ENNIATIN A ($C_{24}H_{42}O_6N_2$). Colourless crystals, m.p. 121–122°, $[\alpha]_D^{20} = -91°$. Soluble in various organic solvents; almost insoluble in water. Structure XXVIII has been proposed. N-methyl-isoleucine is obtained by acid hydrolysis and may be identified on a paper chromatogram. Inhibits growth of acid-fast organisms (*Myc. phlei, M. tuberculosis, M. paratuberculosis*) in the range 1–2 μg./ml.; *B. subtilis* at 3 μg./ml.; *Staph. aureus* at 6 μg./ml.; but *B. coli* is unaffected by 1000 μg./ml. Rapidly destroyed under alkaline conditions. Produced by *Fusarium oxysporum, F. avenaceum, F. sambucinum, F. lateritium* and *F. scirpi* (C) (116, 199–203).

ENNIATIN B ($C_{23}H_{38}O_6N_2$). Colourless crystals, m.p. 173–175°, $[\alpha]_D^{21} = -107°$. Soluble in various organic solvents, including petrol ether; almost insoluble in water. Structure XXVIII has been proposed. N-methyl-valine is obtained by acid hydrolysis and may be identified on a paper chromatogram. Enniatin B inhibits growth of *Mycobacterium phlei* at 3 μg./ml., and *M. paratuberculosis* at 5 μg./ml. Rapidly destroyed in alkali. Produced by *Fusarium oxysporum, F. avenaceum, F. scirpi, F. sambucinum* and *F. lateritium* (116, 199–203).

ENNIATIN C. A name first given (199) to an antibiotic isolated from a strain of *Fusarium oxysporum,* at first believed to be pure ($C_{22}H_{38}O_6N_2$, m.p. 152–153°) but subsequently shown (203) to be a mixture of enniatins A and B.

The name was subsequently applied (202) to an enniatin, never actually isolated, isomeric with enniatin A but yielding N-methyl-leucine on acid hydrolysis instead of N-methyl-isoleucine. Structure XXVIII. Produced by *F. scirpi, F. sambucinum, F. lateritium* and *F. oxysporum.*

EXPANSINE. See PATULIN.

FREQUENTIC ACID. A substance has been described under this name (102) and was said to possess the following properties: $(C_5H_5O_3)n$?, m.p. 155°, optically inactive yellow crystals; gives a deep green colour with ferric chloride; weakly antibacterial, preventing growth of certain Gram + bacteria in broth in concentra-

tions of 125–2000 μg./ml.; produced by *Penicillium frequentans* (C). This substance has since been shown (125*a*) to be identical with the previously described citromycetin (q.v.).

FREQUENTIN ($C_{14}H_{20}O_4$). Colourless needles, m.p. 128°, $[\alpha]_D^{26} = +68°$. Gives a magenta colour with ferric chloride. Soluble in acetone, chloroform, ethyl alcohol and benzene; slightly soluble in carbon tetrachloride and water. It is antifungal, preventing germination of *Botrytis allii* spores at 2.5 μg./ml., but only moderately antibacterial. Produced by some strains of *Penicillium frequentans* (C) (91*a*).

FRUCTIGENIN. Molecular formula uncertain ($C_{26}H_{44\text{-}46}O_7N_2$). Optically active, colourless crystals, m.p. 129°. Soluble in organic solvents, including petrol ether; sparingly soluble in water. Bacteriostatic in broth to *Mycobacterium phlei* at 3.5–5 μg./ml., to *Staph. aureus* at 37.5 μg./ml. Highly unstable in alkaline solution but heat stable in neutral and acid solution. Produced by *Fusarium fructigenum* (C) (80).

FUMIGACIN. See HELVOLIC ACID.

FUMIGATIN ($C_8H_8O_4$). Optically inactive, maroon needles, m.p. 116°. Purple-black colour with ferric chloride in alcohol, changing to light brown on addition of water. With conc. H_2SO_4 a brown colour is given, changing to cherry red in two minutes and intense purple in 30 minutes. Soluble in acetone, ether, chloroform, benzene and ethanol; slightly soluble in petrol ether; fairly soluble in water. The structure has been shown to be 3-hydroxy-4-methoxy-2:5-toluquinone (VII) (16). Inhibits *Staph. aureus* in broth at 20 μg./ml., *B. coli* at 100 μg./ml.; occasional strains of *Staph. aureus, B. anthracis* and *Vibrio cholerae* are more sensitive. Produced by *Aspergillus fumigatus* (C) in yields up to 150 mg./l. (6, 16, 184).

FUSARIC ACID ($C_{10}H_{13}NO_2$). Is 5-*n*-butylpyridine-2-carboxylic acid (Structure XXI). Weakly bacteriostatic, inhibiting growth of *Staph. aureus, S. typhi* and *B. coli* at 1000 μg./ml. A metabolic product of the rice-pathogen *Gibberella fujikuroi* (275).

FUSCIN ($C_{15}H_{16}O_5$). Optically inactive, shining, diamond-shaped orange plates, m.p. (decomp.) 230°. Gives a purplish-red colour with ferric chloride in ethanol; intense purple solution in alkali. Soluble in all organic solvents except petroleum ether;

sparingly soluble in water but freely soluble in alkalis. Its properties are suggestive of a quinonoid structure. Bacteriostatic to Gram + bacteria in the range 1–10 μg./ml., to Gram – bacteria in the range 10–200 μg./ml.; only slightly active against Mycobacteria. Found in culture filtrates from *Oidiodendron fuscum*, particularly if the solution is aerated after removal of the mycelium (175).

GENTISYL ALCOHOL ($C_7H_8O_3$). Optically inactive, colourless needles, m.p. 100°. Gives an evanescent blue colour with aqueous ferric chloride, changing to yellow. Soluble in water, ethanol and ether; slightly soluble in benzene and chloroform; insoluble in petrol ether. Structure XII (2:5-dihydroxybenzyl alcohol). Weakly antibacterial, inhibiting growth of *Staph. aureus* at 2000 μg./ml. Produced by *Penicillium urticae* and *P. divergens* (C). In both cases it is produced together with patulin, and in *P. urticae* the yields of patulin and gentisyl alcohol are in the ratio 2:1 (gentisyl alcohol up to 600 mg./l. of culture filtrate) (18, 26, 100).

GEODIN ($C_{17}H_{12}O_7Cl_2$). Pale yellow needles, m.p. (decomp.), 235°, $[\alpha]_{5461}^{20} = +179°$. In alcoholic solution gives a dirty green colour with ferric chloride, changing to brown. Soluble in chloroform, acetone and ethanol; sparingly soluble in benzene and ether; insoluble in water or petrol ether. Structures XXIV and XXV have been proposed. It is antibacterial, Gram + bacteria being most sensitive; *Staph. aureus* is inhibited in broth at 16–64 μg./ml. Produced by *Aspergillus terreus* (67, 76, 166, 208).

GLADIOLIC ACID ($C_{11}H_{10}O_5$). Optically inactive, long colourless silky needles, m.p. 160°. With strong ammonia it gives a deep green colour, turning to red in 12 hours. Soluble in most organic solvents except petrol ether; moderately soluble in hot water. The structure proposed earlier (4-methyl-3-methoxy-2-carboxyphenylglyoxal) is not correct (125). It is moderately antibacterial (*Staph. aureus* is inhibited in broth at 250 μg./ml.) and antifungal (spore germination inhibited—*B. allii* at 8 μg./ml., *Fusarium graminearum* at 0.9 μg./ml., *Trichoderma viride* at 250 μg./ml.). It is stable in aqueous solution in the range pH 3–8 but is rapidly inactivated in the presence of certain amino-acids or in the presence of ammonium salts above pH 5. It is

produced by *Penicillium gladioli* (C), yields of 300 mg./l. being obtained (47, 50).

GLIOCLADIUM ANTIBIOTICS. A crude orange-coloured antibiotic substance has been isolated from culture filtrates of a species of *Gliocladium,* approximating to *G. roseum,* in yields of 1g./l. Three pure substances have been obtained from the crude material: gliorosein ($C_{10}H_{14}O_4$), colourless crystals, m.p. 48°; aurantioglioclad in ($C_{10}H_{12}O_4$), orange crystals, m.p. 63°; rubroglioclad in ($C_{20}H_{26}O_8$), red crystals, m.p. 74°. These are soluble in organic solvents, including petrol ether, and in water. They are moderately antibacterial, preventing growth of *B. subtilis* and *B. coli* in broth at 400 μg./ml. (54).

GLIOTOXIN ($C_{13}H_{14}N_2S_2O_4$). Colourless needle-shaped crystals, begin to decompose at 165° and melt at about 185°, $[\alpha]_D^{19} = -230°$. Freely soluble in chloroform, dioxane and pyridine; less soluble in ethanol and benzene; only slightly soluble in water. The structure of gliotoxin is at present a little uncertain; XXIII has been proposed. Gliotoxin is antibacterial (growth in broth inhibited at the following concentrations: *Staph. aureus,* 0.25–3 μg./ml.; *B. coli,* 10–20 μg./ml.; *Mycobacterium tuberculosis,* 1 μg./ml.) and antifungal (spore germination inhibited at the following concentrations: *Botrytis allii,* 4 μg./ml., *Fusarium caeruleum,* 2 μg./ml., *Aspergillus niger,* 30 μg./ml.). Acid aqueous solutions are fairly stable, but in neutral or alkaline solutions inactivation is rapid. It is produced (C) by *Trichoderma viride, Aspergillus fumigatus, Penicillium terlikowskii* and *P. cinerascens;* yields as high as 100 mg./l. may be obtained (43, 55, 143, 171, 240–242, 264).

GLUTINOSIN ($C_{48}H_{60}O_{16}$). Thin colourless plates, darkens but does not melt at 300°, $[\alpha]_D^{20} = +54°$. Soluble in chloroform, ether, *n*-butanol and petrol ether; less soluble in ethanol; only slightly soluble in water. Glutinosin is not markedly antibacterial but is highly antifungal (spore germination inhibited at the following concentrations: *Botrytis allii,* 0.8 μg./ml.; *Mucor mucedo,* 1.6 μg./ml.; *Trichoderma viride,* 7.5 μg./ml.). Aqueous solutions are fairly stable in the range pH 3–7.6 but more alkaline solutions are less stable. It is produced by *Myrothecium verrucaria* (C) and can be obtained in yields of 100 mg./l. (49, 57, 61).

GRISEOFULVIN ("Curling-factor") ($C_{17}H_{17}O_6Cl$). Colourless crystals, m.p. 218–219°, $[\alpha]_{5461} = +417°$. Moderately soluble in chloroform; less soluble in benzene, ethanol and dioxane; only slightly soluble in water. Structures XIX and XX have been proposed, but it is doubtful that either is correct (127). Griseofulvin causes abnormal morphogenesis and stunting of many chitin-walled fungi, *Botrytis* spp. being specially sensitive, but cellulose-walled fungi (Oomycetes) are unaffected (44). Aqueous solutions are stable in the range pH 3–8.8. It is produced by *Penicillium griseofulvum, P. urticae* (*patulum*) and *P. nigricans* (*janczewskii*) (C & M). Yields of 200 mg./l. may be extracted from culture filtrates and an approximately equal quantity from the mycelium (48, 51, 126, 187).

HELVOLIC ACID (FUMIGACIN) ($C_{32}H_{44}O_8$). Colourless needle-shaped crystals, m.p. 212°, $[\alpha]_D^{25} = -132°$. Soluble in most organic solvents except petroleum ether; insoluble in water but freely soluble in alkalis. Highly inhibitory to Gram + bacteria, growth of *Staph. aureus* in broth being prevented by 1 μg./ml.; *Myc. tuberculosis* is inhibited by 100 μg./ml. Negligible bactericidal action. Aqueous solutions are stable in the range pH 2–10. Produced by *Aspergillus fumigatus* (C) (73, 171, 236).

HERQUEIN ($C_{19}H_{20}O_8$ (?)). Yellowish-brown crystals, m.p. (decomp.) 129°. Soluble in ethanol, chloroform and ether; insoluble in petrol ether; only slightly soluble in water, giving a yellow solution showing a green fluorescence on addition of alkali. Active against Gram + and acid-fast organisms; *Staph. aureus* and *Vibrio cholerae* are inhibited in broth at 400 μg./ml. Thermostable in aqueous solution in range pH 4.8–7, rather less stable at pH 8, rapidly inactivated at pH 2. Produced by *Penicillium herquei* (C) (63, 102).

HIRSUTIC ACID N. Has not been obtained in an unequivocally pure state; it is colourless. Soluble in most organic solvents and in hot water. Moderately antibacterial; certain Gram + organisms are inhibited at 25–100 μg./ml., Gram – organisms being less sensitive. Aqueous solutions are unstable at pH 6.5–7. Produced by *Stereum hirsutum* (C). The fungus first forms a biologically inactive compound—hirsutic acid C ($C_{15}H_{20}O_4$?) m.p. 179.5°, $[\alpha]_D^{20} = +11.9°$, colourless prisms, which is enzymatically changed into hirsutic acid N (131).

HUMICOLIN. Has been obtained as a yellow oil from culture filtrates of *Aspergillus humicola.* Soluble in organic solvents, including petrol ether, but only slightly soluble in water. Gives a brown colour with ferric chloride in alcoholic solution. Highly antifungal, preventing spore germination at the following concentrations: *Botrytis allii,* 1.6 μg./ml.; *Penicillium expansum,* 6.25 μg./ml.; *Trichoderma viride,* 12.5–25.0 μg./ml. Not appreciably antibacterial. Aqueous solutions are stable in the range pH 3.5–4.5 but unstable above, being rapidly inactivated at room temperature at pH 7 (91).

HYDROXYASPERGILLIC ACID ($C_{12}H_{20}N_2O_3$), m.p. 149°, $[\alpha]_D^{24} = +42°$. Produced, together with aspergillic acid, by *Aspergillus flavus* when grown on brown sugar media. Has one-tenth the antibacterial activity of aspergillic acid (172). Chemical relationship to aspergillic acid has been demonstrated (98).

ILLUDIN M ($C_{15}H_{20}O_3$). Colourless crystals, m.p. 130–131°, $[\alpha]_D^{20} = -126°$. Soluble in most organic solvents, less so in water. Antibacterial, inhibiting *Mycobacterium smegmatis* and *Myc. tuberculosis* at 1 μg./ml. and *Staph. aureus* at 250 μg./ml. Antifungal, inhibiting *Memnoniella echinata* and *Penicillium notatum* at 16 μg./ml. Produced by *Clytocybe illudens* (C) in yields of 300 mg./litre (4*a*).

ILLUDIN S ($C_{15}H_{20}O_4$). Colourless crystals, m.p. 124–125°, $[\alpha]_D^{20} = -165°$. Fairly soluble in many organic solvents and in water. Antibacterial, inhibiting several Gram + and acid-fast bacteria in the range 4–30 μg./ml. Produced by *Clytocybe illudens* (C) in yields of 40 mg./litre (4*a*).

INOLOMIN. A crude preparation has been obtained from *Inoloma traganum* (S) active against certain Gram + bacteria (107).

JAVANICIN ($C_{15}H_{14}O_6$). Optically inactive, copper-red laths, m.p. (decomp.) 208°. Gives a deep violet colour with sodium hydroxide. Soluble in ether and benzene; almost insoluble in petrol ether; only slightly soluble in water but readily soluble in sodium carbonate solution. Partial structure X has been proposed. Antibacterial against Gram + and acid-fast organisms, inhibiting concentrations being: *Staph. aureus* and *Myc. phlei,* 2.5 μg./ml.; *Myc. tuberculosis,* 10–20 μg./ml. Weakly antifungal. Neutral aqueous solutions are the most stable. Produced by *Fusarium*

javanicum (C) along with another pigment, oxyjavanicin (10, 11).

KOJIC ACID ($C_6H_6O_4$). Optically inactive, colourless prisms, m.p. 158°. Gives an intense red colour with ferric chloride. Soluble in water, alcohol and ethyl acetate; almost insoluble in benzene. Structure II. Kojic acid is an antibacterial of low activity, Gram + and Gram – organisms being inhibited at about 2000 μg./ml. It is not appreciably antifungal (163). Aqueous solutions are thermostable in the range pH 3–7.5. Produced (C) by *Aspergillus* spp. in the *wentii, tamarii* and *flavus-oryzae* groups in very high yield; also by *Penicillium daleae* (28, 102, 119, 123, 141, 169, 272).

LACTAROVIOLIN ($C_{15}H_{14}O$). Red-violet pigment, m.p. 53°. Soluble in most organic solvents, including petrol ether. Nearly insoluble in water. Inhibits *Mycobacterium tuberculosis* at 60 μg./ml. Extracted from *Lactarius deliciosus* (S) (147, 263, 264).

LATERITIIN–I ($C_{26}H_{46}O_7N_2$). Optically active, colourless, m.p. 121–122°. Probably identical with enniatin A (82, 261). Soluble in organic solvents, including petrol ether; sparingly soluble in water. Bacteriostatic in broth to *Mycobacterium phlei* at 3–5 μg./ml., to *Staph. aureus* at 20 μg./ml. Highly unstable in alkaline solution but thermostable in neutral and acid solution. Produced by *Fusarium lateritium* (C) (11, 80, 82).

LATERITIIN–II ($C_{26}H_{46}O_7N_2$). Optically active, colourless, m.p. 125°. Solubility as for lateritiin–I. Bacteriostatic in broth to *Mycobacterium phlei* at 3–6.25 μg./ml., to *Staph. aureus* at 25 μg./ml. Stability as for lateritiin–I. Produced by *Fusarium lateritium* (C) (a distinct strain from that producing lateritiin–I) (11, 80, 82).

LINOLEIC ACID ($C_{18}H_{32}O_2$). Was found to be responsible for the antibacterial activity of extracts of the mycelium of *Penicillium crustosum* (164, 165). The antibacterial activity of unsaturated fatty acids has been appreciated for some time (20, 154).

LYCOMARASMIN ($C_9H_{15}O_7N_3$). White microcrystalline powder, m.p. (decomp.) 227–229°, $[\alpha]_D = -42$ to $-48°$. Sparingly soluble in water, but dissolves in dilute acid or alkali. Lycomarasmin is a peptide, and structures XXVI and XXVII have been proposed,

of which XXVII best accounts for its properties. Lycomarasmin causes wilting of higher plants but is also inhibitory to bacteria (270) and fungi (germination of *Botrytis allii* spores is inhibited at 50 μg./ml. (46)). Produced by *Fusarium bulbigenum* var. *lycopersici* (C) in yields of about 100 mg./1. (114, 116, 197, 198, 203, 270, 271).

MARASMIC ACID ($C_{16}H_{20}O_4$?). Long white needles, m.p. 174–175°, $[\alpha]_D^{25} = +176°$. Gives no colour with ferric chloride; reduces Fehling's solution. Soluble in acetone, ethanol, ether, chloroform and water, but insoluble in hexane and petrol ether. Markedly inhibitory to Gram+ bacteria (*B. subtilis* inhibited in broth at 1 μg./ml.; *Staph. aureus* at 2 μg./ml.) and antifungal (many fungi inhibited in the range 16–64 μg./ml.). Produced by *Marasmius conigenus* (C) in yields of 100 mg./1. (149).

MARASMIUS RAMEALIS ANTIBIOTICS. A brief description has been given of the isolation of two antibacterial substances, one being volatile and the other non-volatile but ether-soluble, by *Marasmius ramealis* (C) (22).

MARASMIUS GRAMINUM ANTIBIOTICS. Two active agents have been isolated from culture filtrates. One of these has been isolated as red crystals, m.p. 84.5°, soluble in ether, chloroform and ethanol, sparingly soluble in petrol ether and water. Gives a yellow solution in water, turning violet on addition of alkali. Moderately inhibitory to *Staph. aureus* and other Gram+ bacteria (21).

MELLEIN ($C_{10}H_{10}O_3$). Optically inactive, colourless needles, m.p. 58°. Soluble in chloroform, ether, acetone and alcohol; almost insoluble in water and petrol ether. Structure XV. Weakly inhibitory to *Staph. aureus*. Produced by *Aspergillus melleus* and *A. ochraceus* (C) in yields of 200 mg./1. (64, 181, 182, 273, 274).

4-METHOXY-2:5-TOLUQUINONE ($C_8H_8O_3$). Optically inactive, golden-yellow plates or clusters of short prisms, m.p. 170–172°. Solutions in alkali darken rapidly to purplish-brown; gives a bright orange-red solution in conc. H_2SO_4. Soluble in ether and chloroform, less soluble in ethanol and water. Structure VI. Moderately antibacterial (*B. subtilis* inhibited at 4 μg./ml. and *B. coli* at 64 μg./ml.) and antifungal (*Aspergillus niger* inhibited at 32 μg./ml.; *Myrothecium verrucaria* at 64 μg./ml. and *Peni-*

cillium notatum at 128 μg./ml.) (4). Produced by *Coprinus similis* and *Lentinus degener* (C) (4).

6-METHYL SALICYLIC ACID ($C_8H_8O_3$). Optically inactive, long colourless needles, m.p. 170–171°. Gives an intense purple colour with ferric chloride. Soluble in ether and alcohol; moderately soluble in hot water or chloroform; nearly insoluble in cold water. Structure XIII. It is moderately antifungal (46), preventing germination of *Botrytis allii* spores at 6.25 μg./ml. Produced by *Penicillium claviforme, P. griseofulvum* and *P. urticae* (*flexuosum*) in considerable yields (C) (5, 124, 186, 206).

MYCELIANAMIDE ($C_{22}H_{28}O_5N_2$). Colourless shining leaflets, m.p. (decomp.) 170–172°, $[\alpha]_{5461}^{19} = -182°$. Gives a deep reddish-brown colour with very dilute ferric chloride. Sparingly soluble in cold ethanol, chloroform, ether and benzene; readily soluble in acetone and dioxane; insoluble in water but easily soluble in sodium carbonate solution. Antibacterial, Gram + bacteria being inhibited at 20–50 μg./ml.; Gram – bacteria unaffected. Antifungal, germination of *Botrytis allii* being inhibited at 25 μg./ml. Produced by *Penicillium griseofulvum* (M) (185, 187).

MYCOPHENOLIC ACID ($C_{17}H_{20}O_6$). Optically inactive, colourless needles, m.p. 140°. With ferric chloride it gives a bright blue colour in ethanol, bluish-violet in aqueous solution. Very soluble in alcohol; moderately soluble in ether and chloroform; sparingly soluble in water. Structure XVIII has recently been proposed (25). Antibacterial, being more effective against Gram + than Gram – bacteria. Using small inocula *Staph. aureus* may be inhibited at about 15 μg./ml., but if the size of the inoculum is increased, as much as 1000 μg./ml. may be needed. Antifungal, showing extreme specificity; growth in agar of *Stereum purpureum* and a number of dermatophytes was inhibited by 12.5 μg./ml.; *Phytophthora erythroseptica* by 50 μg./ml. but many fungi were unaffected by 200 μg./ml. (104, 121). Produced by species of the *Penicillium brevicompactum* series and possibly also by *P. viridicatum* (C) (3, 63, 77, 102, 259–261).

NEBULARINE. Has been obtained as a crude concentrate from *Clitocybe nebularis* (S). Soluble in water and methanol; insoluble in ether and chloroform. Active against *Mycobacteria.* Thermostable (159).

NEMOTIN. A neutral substance, obtained only in solution because a brown inactive product is formed on drying. $[\alpha]_D^{23}$ = +202°. Soluble in ether, chloroform and acetone; very sparingly soluble in water and hexane. Antibacterial, inhibiting growth in broth at following concentrations: *Staph. aureus,* 2 μg./ml.; *B. subtilis,* 0.06–0.125 μg./ml.; *B. coli,* 16–64 μg./ml. Antifungal, preventing spore germination at the following concentrations: *Aspergillus niger, Myrothecium verrucaria,* 4 μg./ml.; *Stemphylium consortiale,* 16–64 μg./ml. Acid aqueous solutions are stable, but at pH 6.0 or above nemotin changes into another biologically active substance (nemotin A), showing a quite distinct UV absorption spectrum. Produced by *Poria corticola* and *P. tenuis* (C) (150).

NEMOTINIC ACID. Has not been obtained other than in solution. $[\alpha]_D^{23}$ = +270°. Soluble in ether, chloroform, alcohol and acetone; slightly soluble in water and hexane. It is antibacterial, inhibiting growth in broth at the following concentrations: *Staph. aureus,* 1 μg./ml.; *B. subtilis,* 0.25 μg./ml.; *B. coli,* 64–125 μg./ml. Moderately antifungal, preventing spore germination at the following concentrations: *Aspergillus niger,* 125 μg./ml.; *Myrothecium verrucaria,* 250 μg./ml.; *Stemphylium consortiale,* 250 μg./ml. Aqueous solutions are stable at room temperature for one hour in the range pH 3.6–9.4, but at pH 12.6 considerable change takes place. Produced by *Poria corticola* and *P. tenuis* (C) (150).

NOTATIN (PENATIN, PENICILLIN B, CORYLOPHILIN). A flavoprotein enzyme, a glucose aerohydrogenase or glucose oxidase, powerfully bactericidal in the presence of glucose and oxygen, owing to the production of hydrogen peroxide. A partially purified preparation, in the form of a buff powder, has been isolated from culture filtrates from *P. chrysogenum, P. notatum* and *P. resticulosum,* in yields of 30–40 mg./l. It is probably produced by other moulds. Thermolabile and decomposes in solution at room temperature below pH 2 or above pH 8 (84, 85).

NUDIC ACID A ($C_{14}H_{20}O_3$ (?)). Colourless glistening needles or plates, m.p. 123.5°. Decolourises neutral aqueous permanganate and bromine water. Soluble in most organic solvents, including hexane; nearly insoluble in cold water but freely soluble in sodium carbonate solution. Generally bacteriostatic, inhibiting

growth of *Staph. aureus* in broth at 125 μg./ml. and of *B. coli* at 500 μg./ml. Heat stable in neutral solution, only slightly unstable in N/10 acid or alkali. Produced along with nudic acid B by *Tricholoma nudum* (C) (102).

NUDIC ACID B ($C_4H_8O_3$ (?)). Small colourless needles, darkening and subliming at 150°. On exposure to sunlight at room temperature for 24 hours the crystals darken, becoming black. Decolourises neutral aqueous permanganate but not bromine water. Soluble in most organic solvents but insoluble in hexane, by which character it may be separated from nudic acid A. More highly active than nudic acid A, growth of *Staph. aureus* being inhibited by about 2 μg./ml., *B. coli* by 4–32μg./ml. Thermostable in neutral and acid solution but not in alkali. Produced by *Tricholoma nudum* (C) (102).

OREGONENSIN ($C_{20}H_{32}O_8$). Optically inactive, white needles, m.p. 82°. Soluble in most organic solvents except petrol ether; sparingly soluble in water. Highly active against Gram + and acid-fast bacteria; *Staph. aureus* is inhibited in broth at 1.25 μg./ml., *Mycobacterium phlei* at 5 μg./ml. Stable at room temperature in the range pH 2–7, but unstable in alkaline solution. Produced by *Ganoderma oregonense* (C) (102).

OXYJAVANICIN ($C_{15}H_{14}O_7$). Optically inactive, red needles with coppery lustre, m.p. (decomp.) 213–214°. Structure XI. It is antibacterial, showing the same activity against *Staph. aureus* as javanicin. Produced by *Fusarium javanicum* (C) (10, 11).

PATULIN (CLAVATIN, CLAVACIN, CLAVIFORMIN, EXPANSINE) ($C_7H_6O_4$). Optically inactive, colourless rhomboid plates, m.p. 111°. It gives a yellow colour with dilute ammonia, reduces Fehling's solution in the cold. Soluble in ether, alcohol and acetone; moderately soluble in water, insoluble in benzene and petrol ether. Structure XIV has recently been proved by synthesis (268, 269). Generally antibacterial, growth in broth being inhibited at the following concentrations: *Staph. aureus*, 6–20 μg./ml.; *S. typhi*, 6 μg./ml.; *B. coli*, 12 μg./ml. It is also antifungal; certain Phycomycetes are sensitive, species of *Pythium* and *Phytophthora* being inhibited at 1–3 μg./ml., but many other fungi are insensitive to 200 μg./ml. Stable in acid and neutral solution, unstable in alkali. Produced by a variety of fungi, including *Penicillium urticae, P. expansum, P. claviforme, P. melinii, P.*

divergens, P. terrestre, P. novae-zeelandiae, Aspergillus clavatus, A. giganteus, A. terreus and *Gymnoascus* sp. Extracted from culture filtrates in yields as high as 1.4 g./1. (9, 23, 29, 71, 72, 99, 179, 180).

PENICILLIC ACID ($C_8H_{10}O_4$). Optically inactive colourless crystals, m.p. 86° (anhydr.) 64–65° (hydrated). No colour with ferric chloride in the cold, orange-brown colour on heating. Reddish-purple colour formed with strong ammonia on standing. Soluble in water, alcohol, ether, benzene and chloroform; insoluble in cold petrol ether. Structure I. Generally antibacterial, growth in broth being inhibited at following concentrations: *Staph. aureus,* 20 μg./ml.; *B. coli,* 10 μg./ml.; *S. typhi,* 30 μg./ml. Slightly antifungal, a concentration of at least 200 μg./ml. being required to inhibit growth, many fungi being unaffected by even higher concentrations. Produced by many species, including *Aspergillus ochraceus, A. quercinus, A. sulphureus, Penicillium thomii, P. baarnense, P. puberulum, P. cyclopium* and *P. roqueforti* (C), and yields may be as high as 2.5 g./1. (3, 30, 146, 188, 190, 211, 212).

PENICILLIN. Outside the scope of this review.

PENICILLIUM SPINULOSUM ANTIBIOTIC. Kondo and Takahashi describe the isolation of a generally antibacterial substance, m.p. 183–185°, white needles, from culture of *P. spinulosum* (155).

PHOENICIN ($C_{14}H_{10}O_6$). Small yellow prisms, m.p. (decomp.) 231–232°. Soluble in chloroform, acetic acid and ethanol; sparingly soluble in water. Aqueous solutions are yellow below pH 1.6, red at pH 1.6–3.6, violet at higher pH levels. Structure IX. It is mildly antibacterial, growth of *Staph. aureus* in broth being inhibited at 1000 μg./ml. Produced by *Penicillium phoenicum* and *P. rubrum* (C) in yields of 0.5 to 1.5 g./1. (63, 88, 102, 112, 205).

PICACIC ACID. Has been isolated in crude form as a pale yellow oil, freely soluble in many organic solvents but only slightly soluble in water. It is weakly antibacterial, a range of Gram + and Gram – bacteria being inhibited by concentrations of 1000–4000 μg./ml. Aqueous solutions are stable in the range pH 2–7 but rapidly inactivated at pH 9. Produced by *Coprinus picaceus* (C) (102).

PLEUROTIN ($C_{20}H_{22}O_5$). Amber or yellow needles, m.p. 209°,

$[\alpha]_D^{23} = -20°$. Gives a blue colour with potassium cyanide. Soluble in chloroform, acetone, ether and ethanol, sparingly soluble in water. Gram + bacteria are sensitive to this substance, growth of *Staph. aureus* and *B. subtilis* in broth being inhibited by 0.2–0.8 μg./ml.; the Gram – *B. coli* requires 500 μg./ml. for inhibition. Aqueous solutions are unstable in light or if heated, particularly above pH 8.5 or below pH 3. Produced by *Pleurotus griseus* (C) in yields of 100–180 μg./ml. (218).

POLYPORENIC ACID A (UNGULINIC ACID) ($C_{30}H_{48}O_4$ or $C_{31}H_{50}O_4$). Snow-white crystals, m.p. 190–193°, $[\alpha]_D^{18} = +70°$. No colour reaction with ferric chloride. Soluble in many organic solvents but only sparingly soluble in chloroform and benzene. Nearly insoluble in water but soluble in sodium carbonate solution. It is specific in its activity; certain anaerobic bacteria are inhibited at 0.3–2 μg./ml., whereas *B. subtilis* and a number of mycelial fungi are unaffected by high concentrations. Produced by *Ungulina* (= *Polyporus*) *betulina* (S) (87, 158).

POLYPORIN. A crude antibiotic isolated from culture filtrates of *Polystictus sanguineus* (39, 40).

POLYPORUS CINNABARINUS ANTIBIOTIC. Has been obtained in crude form by extraction of sporophores of *P. cinnabarinus* with water. It is not soluble in chloroform, carbon tetrachloride or benzene. It inhibits a number of Gram + bacteria at 20 μg./ml., but Gram – bacteria are insensitive (173, 174).

POLYSTICTIN. Has been obtained as a crude yellow powder by extraction of the mycelium of *Polystictus versicolor*. Insoluble in most organic solvents, but soluble in water and aqueous alcohol or aqueous acetone. Its antibacterial activity is highly specific, *Staph. aureus* being one of the few sensitive organisms. Aqueous solutions are heat stable, but unstable above pH 7 (102).

PUBERULIC ACID ($C_8H_6O_6$). Optically inactive, pale cream crystals, m.p. (decomp.) 316°. Gives a brown-red colour and black precipitate with ferric chloride, dissolving to form a reddish solution in excess reagent. Soluble in many organic solvents; slightly soluble in water. Structure IV. Weakly antibacterial, various Gram + and Gram – organisms being inhibited in broth in the range 100–200 μg./ml. Rennerfelt (213) has shown it to be antifungal, inhibiting germination of spores of *Polyporus annosus*

at 10 μg./ml. Produced by *Penicillium puberulum, P. cyclopium, P. martensii* and *P. aurantiovirens* (C) in yields of 100 mg./l. (17, 32, 83, 189).

PUBERULONIC ACID ($C_9H_4O_7$ (83)). Bright yellow prisms, m.p. 298°. Ferric chloride reactions as for puberulic acid. Structure V. Weakly antibacterial, being approximately half as active as puberulic acid. It is also antifungal (213), having one-tenth the activity of puberulic acid (q.v.). Produced by *Penicillium puberulum, P. cyclopium, P. martensii* and *P. aurantio-virens* (C) in yields of up to 60 mg./l. (17, 32, 83, 89).

RAMEALIN. Has been obtained in crude form as a pale yellow oil. It is acidic, decolourises acid and alkaline permanganate but gives no colour with ferric chloride. Soluble in many organic solvents; sparingly soluble in water. It is weakly antibacterial, growth of *Staph. aureus* in broth being inhibited by 400 μg./ml. Aqueous solutions are stable unless alkaline. Produced by *Stereum rameale* (C) (102).

"RED PIGMENT" $(C_5H_4O_2)_n$. Optically inactive, glistening red crystals, darkening at 120° but not melting at 380°. Gives a green solution in conc. H_2SO_4, orange in conc. HNO_3, deep crimson in conc. HCl. Readily soluble in hot ethanol, slightly soluble in cold benzene, dioxane, ethanol and water. Antibacterial (a range of Gram + and Gram – bacteria are inhibited at 10–100 μg./ml.) and highly antifungal (*B. allii* spore germination is inhibited at 0.4 μg./ml.). Aqueous solutions are fairly stable at pH 3.5, unstable at higher pH. Produced by *Penicillium albidum* (C) in yields of 8 mg./l. (90).

ROSEIN II ($C_{19}H_{28}O_3$). Colourless fibrous needles, m.p. 186°, $[\alpha]_D^{23} = +6°$. Soluble in most organic solvents, less so in water. Not antifungal but moderately inhibitory to *B. subtilis.* Isolated from mycelium of *Trichothecium roseum* (111). Identical with rosonolactone of other workers (220).

SAMBUCININ ($C_{24}H_{42}O_7N_2$). Optically active colourless crystals, m.p. 85–86°. Soluble in organic solvents, including petrol ether; sparingly soluble in water. Bacteriostatic in broth to *Mycobacterium phlei* at 5–25 μg./ml., to *Staph. aureus* at 25 μg./ml. Highly unstable in alkaline solutions but heat stable in neutral and acid solution. Produced by *Fusarium sambucinum* (C) (80).

SPARASSOL ($C_{10}H_{12}O_4$). Optically inactive, colourless needles, m.p. 67–68°. Soluble in acetone, ether and chloroform; less soluble in ethanol, methanol and water. Structure XVI. Antifungal, activity not specified. Produced by *Sparassis ramosa* (C), yields considerable (101, 237).

SPINULOSIN ($C_8H_8O_5$). Optically inactive, purplish-bronze plates, m.p. 201°. Gives a dark brown colour with ferric chloride in ethanol, deep blue colour with conc. H_2SO_4, bluish-purple solution in 2 *N* caustic soda. Soluble in many organic solvents; sparingly soluble in water but soluble in pH 7 buffer. Structure VIII proved by synthesis. Moderately antibacterial, Gram + and Gram – bacteria being inhibited in broth in the range 100–400 μg./ml. Produced by *Penicillium spinulosum, P. cinerascens* and *Aspergillus fumigatus* (C). (6–8, 31, 41, 184).

STIPITATIC ACID ($C_8H_6O_5$). Optically inactive, cream-coloured crystals, m.p. (decomp.) 302–304°. Gives a deep red colour with ferric chloride in aqueous solution. Soluble in ethanol; almost insoluble in benzene and chloroform. Sparingly soluble in water but readily soluble in dilute alkalis. Structure III. Rennerfelt (213) has recently shown that this substance prevents germination of *Polyporus annosus* spores at 100 μg./ml. It does not inhibit germination of *Botrytis allii* conidia at 100 μg./ml. under similar conditions (46). Produced by *Penicillium stipitatum* (C) (27, 83).

SULPHOCHRACEIN. Has been obtained as an impure white powder, moderately soluble in chloroform, ethanol and benzene; less soluble in carbon tetrachloride; almost insoluble in water. It is not antifungal, but is weakly inhibitory (ca. 1000 μg./ml.) to Gram + bacteria. Stable in aqueous solution at room temperature at pH 7–8 but rapidly inactivated at pH 5.5 or below. Inactivated by cysteine. Produced by *Oospora sulphurea-ochracea* (C) in yields of 30 mg./l. (132).

TARDIN. Molecular formula uncertain. Pale yellow oil, $[\alpha]_D^{20} = -11.4°$. Gives a purple colour with ferric chloride in alcohol on warming. Soluble in most organic solvents, sparingly soluble in water. Antibacterial, Gram + organisms being more sensitive than Gram – (e.g., growth in broth inhibited as follows: *Staph. aureus,* 4 μg./ml., *Salmonella enteritidis,* 1000 μg./ml.). Aqueous solu-

tions stable in the range pH 2–8. Produced by *Penicillium tardum* (C) (36).

TERREIC ACID ($C_7H_6O_4$ (?)). Colourless plates, m.p. 120–121°. Gives a deep red colour with ferric chloride. Soluble in many organic solvents; sparingly soluble in water. Generally antibacterial, many Gram + and Gram – bacteria being inhibited in the range 100–200 μg./ml.; *S. typhi* was particularly sensitive, 12 μg./ml. being inhibitory. Thermostable except in alkaline solution. Produced by occasional strains of *Aspergillus terreus* in low yield (C) (102).

TRICHOTHECIN ($C_{19}H_{24}O_5$) (110*a*). Slender colourless needles, m.p. 118°, $[\alpha]_D^{18} = +44°$. Readily soluble in most organic solvents; slightly soluble in water. Not appreciably antibacterial but markedly antifungal; spore germination is inhibited at the following concentrations: *Botrytis allii*, 6 μg./ml.; *Penicillium digitatum*, 1.25 μg./ml. Aqueous solutions are stable in the range pH 1–10. Produced by *Trichothecium roseum* (C) (108, 109, 110).

UNGULINIC ACID. See POLYPORENIC ACID A.

USTIZEAIN (A AND B). Names given to two comparatively stable, lipoid-soluble antibiotics produced by *Ustilago zeae* (C). Ustizeain A is antibacterial, inhibiting a number of Gram +, Gram – and acid-fast bacteria. Ustizeain B is antifungal.

VIRIDIN ($C_{19}H_{16}O_6$) (231). Fine colourless needles, m.p. (decomp.) 208–217°, $[\alpha]_D^{20} = -213.4°$. Soluble in chloroform; less soluble in ethanol, methanol and benzene; almost insoluble in water. It is antifungal, but specific in action; shows very high toxicity to some fungi (e.g., germination of *Botrytis allii* spores is inhibited at 0.005–0.01 μg./ml.), other fungi being much more resistant (e.g., *Trichoderma viride*, inhibited at 50 μg./ml.). It is not antibacterial. Aqueous solutions are stable at pH 3 but less stable at higher pH, inactivation being rapid at pH 7 at room temperature. Produced by certain strains of *Trichoderma viride* (C), yields of 50 μg./ml. being obtained. On occasion an isomeric substance (β-viridin) is isolated together with the viridin described above, though in much smaller yield. This has an identical UV absorption spectrum but differs in m.p. (140°) and optical activity ($[\alpha]_D^{20} = -50.7°$). It is less antifungal, inhibiting *B. allii* at 0.16 μg./ml. (52, 60, 231).

GENERAL DISCUSSION

A number of general observations may be made on the factual material systematically summarised in the foregoing treatment.

The survey of antibiotic production in different groups of fungi shows how very unevenly these groups have been studied. The Moniliales (Fungi Imperfecti) and Agaricales (Basidiomycetes) have received far more attention than other groups; the Phycomycetes, Ascomycetes, the form-orders Sphaeropsidales and Melanconiales of the Fungi Imperfecti and certain orders of the Basidiomycetes remain as almost untouched fields of investigation.

We may conclude that, as a broad general principle, closely related species tend to produce the same antibiotics or groups of antibiotics, but that different genera or families tend to produce different antibiotics. This is generally true, in spite of such well-known exceptions as patulin, which is produced by species in several distinct genera, and in spite of the close chemical relationship seen in certain antibiotics produced by species widely separated taxonomically, e.g., spinulosin produced by *Penicillium spinulosum* (Fungi Imperfecti) and 4-methoxy-2: 5-toluquinone produced by *Coprinus similis* (Basidiomycetes). It follows from this that those groups of fungi, mentioned above, which have received little attention in the past, might be particularly fertile ground in the search for new antibiotics of novel chemical structure and biological properties. But this observation should not be allowed to obscure the fact that, even in those groups as yet studied most intensively, much more remains to be done.

There is no doubt that the vast majority of those fungi known to produce antibiotics are characteristically soil fungi. This has been pointed out by several authors, but the present writer, on a previous occasion (45), suggested that production of antibiotics is particularly characteristic of the soil habitat and that it is more common in these fungi than, for instance, those characteristically parasitic on the aerial parts of higher plants. This survey has shown that this point of view may need revision. The "fungi characteristically parasitic on the aerial parts of higher plants" in most cases are species of Ascomycetes, species in the orders Melanconiales and Sphaeropsidales of the Fungi Imperfecti (so aptly grouped by Grove (128) as "stem and leaf fungi"), and species of the orders Uredinales and Ustilaginales of the Basidi-

omycetes; in other words these fungi are precisely those which have been least studied, and until this has been done no comparison can be made with assurance of the antibiotic activity of soil fungi and of leaf and stem fungi. Moreover, the discovery that certain antibiotics produced by plant-parasitic fungi, notably lycomarasmin and alternaric acid, may play a part in the production of characteristic disease symptoms, increases the potential value of study of these groups of fungi.

Some 96 antibiotics have been mentioned in this review. Many of them have been obtained only as crude concentrates, and only 57 have been obtained in pure form and well characterised. Of the characterised antibiotics, the Ascomycetes provide one, the Fungi Imperfecti 48 (33 from the genera *Penicillium* and *Aspergillus* alone), and 8 come from the Basidiomycetes. There is little doubt that the lead at present held by the genera *Penicillium* and *Aspergillus* will be shortened in the next few years, and, in particular, that an increasing number of well-characterised antibiotics from the Basidiomycetes is to be expected.

Two properties shared by most of the antibiotics described in this review merit mention. The first is their marked lipoid solubility and low water-solubility. This property greatly simplifies the process of isolation of pure antibiotics from culture filtrates containing them in low concentration. Secondly, most antibiotics whose spectrum of toxicity has been thoroughly studied appear to be generally toxic. The more specific types of toxicity so far observed are of three kinds: (*a*) specific toxicity to Gram + bacteria, e.g., penicillin and helvolic acid; (*b*) semi-specific toxicity to acid-fast bacteria, Gram + organisms also being affected at somewhat higher doses, e.g., most of the *Fusarium* antibiotics; (*c*) specific toxicity to fungi, e.g., griseofulvin and viridin. It seems likely that other forms of specificity will be found if sought.

The fact that general toxicity is usual raises the interesting question of why organisms producing such antibiotics are not affected. The answer to this question is not a simple one. In some cases the organism producing an antibiotic does appear to be remarkably and inexplicably resistant to the antibiotic which may accumulate in the culture medium without apparent effect; such a case is the production of viridin by *Trichoderma viride*. In other instances the organism is affected, but only to a slight de-

gree, as in the production of griseofulvin by *Penicillium janczewskii*, the hyphae of which usually show the typical abnormal morphology which characterises fungal growth in the presence of griseofulvin. In yet other cases an antibiotic may be very toxic to the organism which produces it; this results in cessation of metabolism and growth, unless the antibiotic is by some means isolated from the fermentation system. Such is seen in the production of gladiolic acid by *Penicillium gladioli*. Gladiolic acid in the undissociated form (i.e., at low pH) is very toxic to *P. gladioli*, but the gladiolic anion (in which form gladiolic acid exists in solutions near or above neutrality) is non-toxic, presumably owing to non-penetration; consequently gladiolic acid accumulates only in cultures where the fermentation also results in the maintenance of a pH near neutrality. This type of mechanism, whereby a fungus escapes from the effects of its toxic metabolic products, is probably common; this review reveals that a high proportion of the antibiotics described are weak acids, so that the physiological advantages which may be derived from the easy change from the lipoid-soluble undissociated form to the water-soluble anion may frequently be enjoyed.

It may be remarked that the conclusions drawn from the considerable quantity of primary data reviewed here are not of outstanding importance. At the present stage of research this is inevitably true. The prospect for the future is fittingly expressed by Florey and his colleagues (102): "Pursuit of fundamental knowledge of microbial antagonism is resulting in the discovery of new substances whose chemical structure and biological specificity are alone of sufficient interest to compensate, in some measure, for the labour and difficulties involved in their isolation. It is also leading to a large accumulation of data, which, though at present unexciting, may some day, like observations on morphology, find a place in a coherent account of the varied activities of living cells".

SUMMARY

1. Fungi are at present known to produce about 96 apparently distinct antibiotics; many of these have been obtained in crude form only and may subsequently be found to be identical with others previously described. Only 57 have been well character-

ised; of these, 48 have been obtained from Fungi Imperfecti, eight from Basidiomycetes and one from Ascomycetes. Many fungi have been shown to produce antibiotics without the active substance having been isolated; this is particularly true of the Basidiomycetes, and an early increase in the number of isolated Basidiomycete antibiotics is to be expected.

2. Neither the numbers of antibiotics so far obtained from each of the main taxonomic groups, nor the number of positive indications of antibiotic activity, is necessarily a reflection of the true potentialities of these groups. This review has demonstrated that far more attention has been paid to fungi in the orders Moniliales (Fungi Imperfecti) and Agaricales (Basidiomycetes) than to any other groups; the apparent superiority of species in these two orders as producers of antibiotics may well be only a reflection of the more detailed study made. In these two orders 51% and 36%, respectively, of the species examined have shown activity. Phycomycetes have been little studied, and it is not possible to assess reliably their potentialities as producers of antibiotics. It is remarkable to find also that two orders of the Fungi Imperfecti —Sphaeropsidales and Melanconiales—have been almost completely neglected.

3. The antibiotics isolated have in most cases been tested against only a limited number of bacteria of medical importance, though recently there has been a growing tendency to examine the antifungal properties of newly isolated antibiotics. It is not possible, because of the restricted information at present available, to assess accurately the range of toxicity of many fungal antibiotics. However, it seems probable that a high proportion are generally toxic substances. Among those with more specific activity, three types of specificity have been observed: (*a*) selective toxicity to Gram + bacteria; (*b*) high toxicity to acid-fast bacteria associated with moderate toxicity to Gram + bacteria and little or none to other microorganisms; (*c*) selective toxicity to fungi.

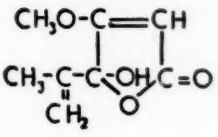
4. Although some antibiotics may show similarities of chemical structure, in general they show no chemical unity as a class, many different types of structure being represented. It is noticeable, however, that nearly all are lipoid-soluble and only sparingly water-soluble, and that a high proportion are acidic.

ACKNOWLEDGMENTS

I am indebted to Miss Joyce Wright and Miss Margaret Radley for assistance in collecting and arranging information from the many sources consulted while preparing this review, to Mr. J. F. Grove for correcting many errors and omissions, and to many other colleagues for helpful criticism of early drafts.

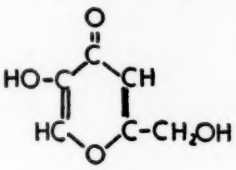
TABLE IV

CHEMICAL STRUCTURE OF ANTIBIOTICS PRODUCED BY FUNGI



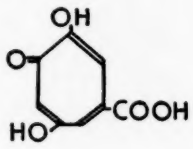
I

Penicillic acid



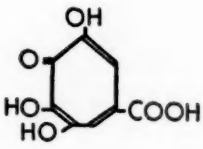
II

Kojic acid



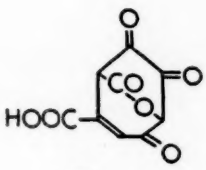
III

Stipitatic acid



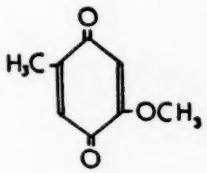
IV

Puberulic acid



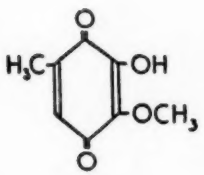
V

Puberulonic acid



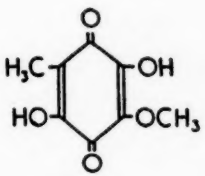
VI

4-Methoxy-2:5-
-toluquinone



VII

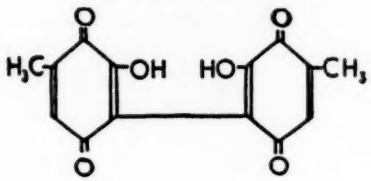
Fumigatin



VIII

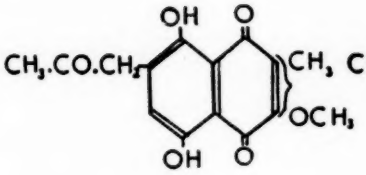
Spinulosin

TABLE IV—(*Continued*)



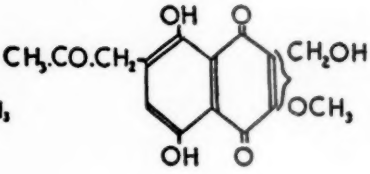
IX

Phoenicin



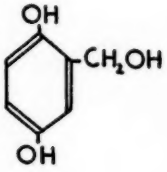
X

Javanicin



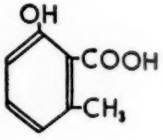
XI

Oxyjavanicin



XII

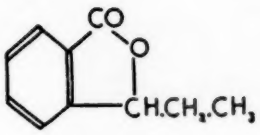
Gentisyl alcohol



XIII

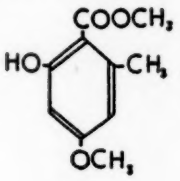
6-Methyl-salicylic acid

TABLE IV—(*Continued*)



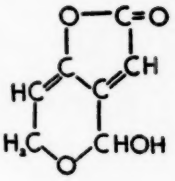
XIV

Patulin



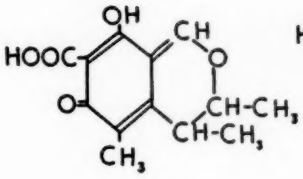
XV

Mellein



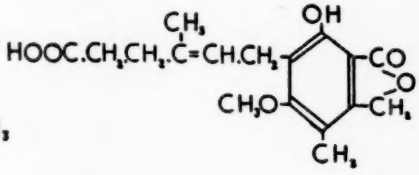
XVI

Sparassol



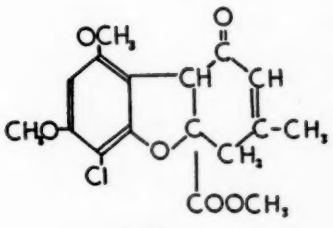
XVII

Citrinin

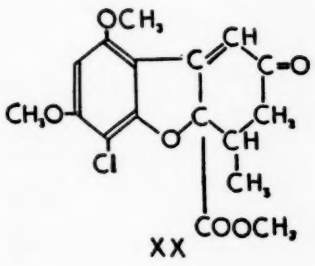


XVIII

Mycophenolic acid



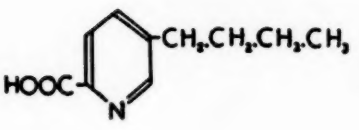
XIX



XX

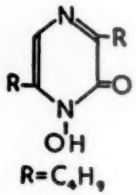
Griseofulvin

TABLE IV—(*Continued*)



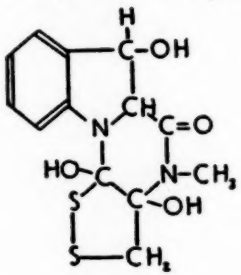
XXI

Fusaric acid



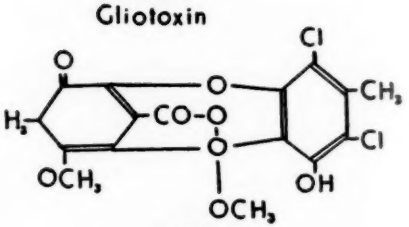
XXII

Aspergillic acid

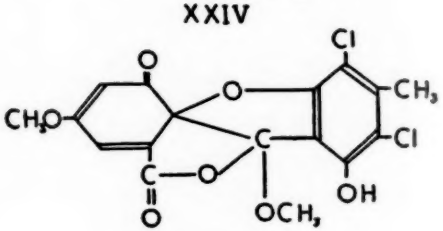


XXIII

Gliotoxin



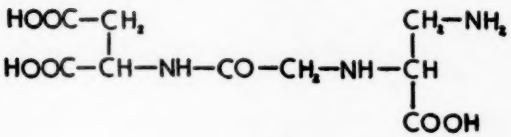
XXIV



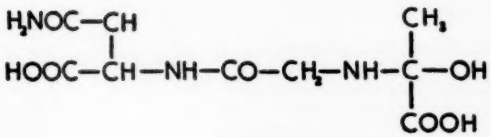
XXV

Geodin

TABLE IV—(*Continued*)

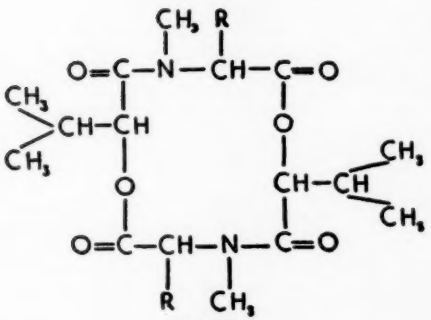


XXVI



XXVII

Lycomarasmin



XXVIII

Enniatin A : R=CH.(CH_3)(C_2H_5)
Enniatin B : R=CH.(CH_3)$_2$
Enniatin C : R=CH_2.CH.(CH_3)$_2$

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AMERICAN COAL-BALL FLORAS

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INTRODUCTION—COLLECTING PROCEDURE

Over seventy years ago W. C. Williamson began his studies of the petrified plants of the British coal fields. Other workers of lasting stature had preceded him in England and on the Continent, and some of those early paleobotanists gained access to comparable sources of information, but for one reason or another none had been able to reveal the full potentialities of the botanical treasures that lay stored in the seams of coal. Possibly Williamson himself did not realize the full effect that his investigations would have on the philosophy of plant morphology and evolution.

It is not my intention to present him as the leading botanist of his time. It is evident, however, that his contributions were not only great in themselves but also made possible the full development of the researches of certain of his countrymen, such as F. O. Bower and D. H. Scott, who took up the thread that he laid down and plowed a broader furrow in the same field. Williamson's name will, however, continue to grow brighter as a landmark in the modern period of paleobotany partly because of the botanical significance of his contributions and partly because he revealed the full importance of a most unique source of fossil plant material—petrifactions that we refer to as " coal balls ". Although many of the plants that have been found in coal balls are now commonly encountered even in elementary textbooks, a firsthand knowledge of the occurrence and nature of the petrifactions is still shared by relatively few paleobotanists.

It will, therefore, be useful to present a brief consideration of their origin, where they are found, their chemical composition, and such points of an introductory nature. While the approach of this review is primarily a botanical one it is not possible or desirable to divorce the plants from their geological setting and the use that they may serve in stratigraphical studies as well as in furthering our knowledge of plant evolution.

Coal balls are aggregations of petrified plant remains that are found in the coal seams of England, Belgium, Holland, Germany, the Donetz Basin of Russia, and in many of the central States of this country. They are representative portions of the plant debris that accumulated in the Upper Carboniferous swamps some 250 million years ago. Here and there small patches of the thousands of square miles of this plant graveyard, extending from Kansas east to China, were petrified with calcium and magnesium carbonate and iron sulphide. This prevented the plants from being crushed and altered chemically into the relatively amorphous coal which surrounds them. The petrifying process, the chemistry of which will be touched upon briefly on a later page, was by no means selective as to the place or quality of the plants which it preserved. The coal balls are sporadic in their occurrence and the contained plants may be badly decayed or exquisitely preserved.

American mining methods are largely responsible for the abundance of material that it is possible to obtain in certain areas. In the coal fields of Indiana, Illinois, Iowa and Kansas considerable quantities of coal are obtained by the open-pit or stripping method. Where a coal seam lies within 50 or 60 feet of the surface, and it is often much closer, the overlying soil and shale or limestone rock is removed by giant electric shovels scooping up 30 or more cubic yards at a time. The coal thus exposed may then be inspected directly, and where petrifactions are present they generally appear as rounded elevations in the coal. Since they will not burn it is necessary to remove them before the coal is marketed. Aside from digging them directly out of the coal, which is a most laborious task, they may be obtained on the mine dumps. Where they occur in exceptional abundance it is not uncommon to find them used as road-building material, and if they have not weathered excessively this constitutes a most convenient collecting ground.

A few instances of especially promising localities may be cited as typical of their occurrence and the entailed collecting problems.

1. For about six years we have made periodic trips to the Pyramid mine located a mile south of Pinckneyville, Illinois. Here coal balls occur fairly frequently, a "nest" of a few hundred pounds being located on almost every visit. On occasions, however, we have wandered along the exposed seam of coal for a half mile or more without finding a trace of them.

2. The largest unit mass of coal balls that we have yet encountered turned up in a strip mine near Boonville in southern Indiana. An aggregation of some 30–40 tons occurred in a single huge lump, so large in fact that mining operations had been diverted around it. The quality appeared to be exceptionally good; the pit superintendent indicated that it was the only "rock" that had been encountered in recent years in the coal, and that continued operations would cover up the coal balls within a few weeks. Under such conditions it was necessary to act immediately and on a rather large scale or not at all. Consequently we hauled away some two tons of specimens and certain of our colleagues followed a similar course. A return visit the following summer revealed that no more "rocks" had been encountered in the meantime. Although one may obtain more specimens here in future years, it is equally possible that none will ever be found again—or if more are encountered a paleobotanist may not be on hand to make a collection.

3. Within recent months we have located a strip mine in southeastern Kansas where coal balls of good quality occur in an abundance that a paleobotanist hopes for but does not often expect. They have been taken out of the coal here by the truckloads, and because they constitute the hardest rock available in the immediate vicinity they have been put to use as road ballast. Many tons of specimens are exposed along the sides of the road leading to one of the pits. The specimens at this locality are also unique in that they contain an exceptionally diverse flora, especially large plant fragments, and of particular significance is the fact that they have weathered in a way that renders possible the identification of many plant organs in the field; thus one can be quite selective in the choice of specimens. Collecting here simply involves walking along the roadside ditch and selecting the more promising material.

4. It is a distinct understatement to say that open-pit mines can be uncomfortably warm in the summer months. Fortunately, somewhat more pleasant collecting grounds occasionally turn up. We have gathered a considerable number of specimens from coal seams exposed by a stream cut. In such localities, rare though they be, there is no cause to remove large quantities at any one time. One such locality in southern Illinois has been visited on numerous occasions by several of us who are studying coal-ball

floras. The owner of the property, although cordial in his way, soon realized that there was some value to be attributed to the "rock" in his coal seam. A small payment thereafter became necessary for the privilege. As a tribute to the patience and cooperation of those who tolerate fossil hunters on their property I may add that in more than 12 years of collecting it is the only instance in which an owner has charged me for the privilege of trespassing. Almost without exception the coal mine operators and employees are openly cooperative and not infrequently will go to some trouble and expense to extend their aid.

Much of the basic information that we possess concerning the origin and chemical composition of coal balls was presented by Stopes and Watson in (98) their comprehensive paper, "On the present distribution and origin of the calcareous concretions in coal seams known as 'coal balls'".

Certain of the results given in their work may be included here, since they answer, within the limits of our present knowledge, questions that frequently are asked concerning these petrifactions. There is conclusive evidence to indicate that the coal balls were formed in situ, that is, that they consist of fragments of the plant debris that had accumulated in the Carboniferous swamps. Many of the coal-ball masses are lenticular, and in some instances plant stems have been found extending from one coal ball to another. Also the size of some is so great as to preclude their having been transported.

The actual process of mineralization is no better known for coal balls than it is for other kinds of petrifactions, and it does not seem appropriate to consider that phase of the subject here. Some reference to their chemical composition is pertinent, however, since it is important in the collection and subsequent treatment of the specimens. On the following page I have given three analyses of coal balls selected from Stopes and Watson's study and a single analysis for an American coal ball presented by Darrah. Perhaps the most significant point is the great variation in the percentages of carbonates, pyrites and organic matter, and these published analyses by no means give the extremes. I have seen coal balls from England with a much higher percentage of pyrites than any of the analyses given by Stopes and Watson; the best specimens, however, contain almost none at all.

	I (p. 193)	VI (p. 193)	VI (p. 194)
Calcium carbonate	51.2	91.1	64.4
Magnesium carbonate	42.8	....	1.8
Ferrous carbonate	2.3	4.0	6.0
Calcium phosphate	0.5	....	....
Iron pyrites	0.3	....	21.9
Silicate of alumina	0.1	1.1	1.2
Carbonaceous matter	1.9	3.6	....

Analyses of English coal balls as given by Stopes and Watson (98).

Sulphur	32.3	Calcium dioxide	14.9
Iron	27.1	Water	1.5
Iron oxide	0.8	Silica	0.7
Calcium oxide	19.6	Organic matter	3.1

Analysis of an American coal ball as given by Darrah (30).

Judging from my own observations of some few thousands of American coal-ball specimens, those that contain any appreciable amount of pyrites consistently yield more poorly preserved plants than those that are petrified with calcium carbonate [1]. This does not mean that an occasional heavily pyritized coal ball may not contain plants that are well worth studying. What it does mean is that while an abundance of "carbonate balls" can be obtained it is pointless to collect those of generally inferior quality, unless a particular horizon contains only the latter, or there are comparable extenuating circumstances.

The highly opaque nature of iron sulphide renders ordinary study by transmitted light impossible; consequently ground thin sections are not satisfactory; and the difficulty involved in dissolving the mineral results in peel preparations of inferior quality. Pyrite is a common petrifying mineral and we are badly in need of techniques that will enable us to more efficiently cope with such fossils.

It may also be noted that the percentage of organic matter in American coal balls falls within a much wider range than is indicated by the published analyses given above. The more highly mineralized specimens contain but little organic matter, while at the other extreme specimens are occasionally encountered which are light brown in color, soft and almost lignitic in texture.

[1] In 1941 Darrah (30, p. 50) noted that "no dolomite occurs in American coal balls". Whether analyses of specimens from more recently discovered deposits will bear this out is not known so far as I am aware.

This brief consideration of the chemistry involved is significant with reference to both field and laboratory studies. First, it is possible to eliminate at the source coal balls that are highly pyritized due to their higher specific gravity. Second, the higher the organic content the shorter the etching time that is required in the preparation of a peel. With different specimens from the same locality this may vary from 30 seconds to two or three minutes, using the same strength acid, to obtain peels of comparable density.

Although ground thin sections may occasionally be helpful, the peel method is ideally suited to these studies and is used almost exclusively in my laboratory. This has been adequately described by Darrah (23) and will be only briefly outlined here.

Our specimens are cut on a slow speed diamond saw equipped with blades up to 20 inches in diameter for the larger coal balls. The more massive ones must be broken with a sledge hammer before cutting. The cut surface is smoothed, first with No. 100 carborundum, then with No. 400. It is next etched in a dilute solution of hydrochloric acid, the time varying with the chemical composition. An optimum thickness of the section is obtained in two or three trials. No particular strength acid is needed, but it is of course important that the same strength be maintained in the stock bottle.

When large numbers of specimens are being handled it is our procedure to study the etched surface under a low power binocular microscope. In this way many worthless specimens or ones containing well-known plant remains are immediately eliminated. Next the etched and dried surface is covered with the peel solution (Darrah, 23, p. 76), allowed to dry overnight, and then removed with the aid of a razor. It may then be studied with a binocular scope using reflected light, or representative portions mounted as may be required. The peels are conveniently stored in envelopes and thus occupy little space.

In this way two or three tons of coal balls constituting an impressive if unattractive, dirty and space-absorbing pile of " rocks " are ultimately reduced to a few small drawers of envelopes.

It is usually possible to determine in the field whether or not a coal ball will contain well-preserved plants, but it is not often possible to know what kinds of plants are present. This statement

is deserving of elaboration. In some localities a single genus may be dominant; for example, *Psaronius* roots constitute 70% or more of the fossils at a place in southern Illinois which is being studied at present. Due to their distinctive appearance in either a weathered or freshly broken surface these are readily recognized, and when a specimen appears to consist almost solely of these roots it is immediately discarded in favor of others which may be expected to present a more diverse assemblage. Where arborescent lycopods were dominant the petrifactions may contain a high percentage of the periderm which constituted the bulk of their stems; likewise the wood of the Cordaitales may occur in large quantities. Such specimens may be weeded out to a considerable extent in the field.

At this point we may appropriately consider some of the variations in the quality of preservation as well as certain exceptional cases in order to emphasize the limitations and potentialities of this mode of fossilization.

We are still quite ignorant of the physical and chemical details of the process of petrification, but we are of course interested in the present account in the results of the process rather than the thing itself. It is known that under not uncommonly encountered anaerobic conditions (Barghoorn, 15, and other references cited there), plant tissues may be rather well preserved for hundreds, thousands and even millions of years without the aid of a petrifying mineral. We do not know why the formation of coal balls occurred so sporadically in both time and space. It is evident that the process was in no way selective as to the relative state of decay of the plant remains so petrified. In some instances we find the most fragile parenchymatous tissues well preserved, and in many cases this probably indicates that petrification followed soon after the deposition of the plants. At the other extreme petrifaction may have taken place after the accumulated plant debris had been altered and closely approximated the relatively amorphous structure of coal. As an example of the latter we recently discovered tremendous quantities of coal balls at an abandoned strip mine in Iowa which, upon being cut open, proved to consist of plants that had decayed to such an extent that cellular details had been almost completely obliterated and the plants consolidated to a near-coal stage.

As examples of moderately good preservation we may find the stems and leaves of ferns, lycopods and pteridosperms nearly perfectly preserved; this statement is applied primarily to cell wall structure, although occasionally cell contents are preserved. Frequently the softer tissues such as phloem, cortex and pith are lost. The arborescent lycopods, for example, are represented to a considerable extent by only their steles and periderm.

A growing number of instances of exceptionally fine preservation serve to carry us over the days when our saws and grinding laps turn up only poor material; a few of these may be briefly referred to. Some years ago a lycopod seed (*Lepidocarpon magnificum,* 11) containing a very nicely preserved gametophyte bearing structures which appear to be archegonia was found in an Illinois specimen. More recently Long (63) has described the gametophyte of *Lagenostoma* in an English coal ball. Among the more beautiful fossils found in the coal balls are fertile fern fronds, belonging to the Marattiaceae and Coenopteridales, and where they were preserved just prior to dehiscence the sporangia are filled with characteristically sculptured spores. An undescribed articulate cone turned up in my laboratory a short time ago in which the contained spores were found to have their nuclei intact and some spores show configurations which seem to represent chromosomes. Dr. John Hall, working in the University of Illinois paleobotanical laboratory, recently showed me sections of *Heterangium* (a seed-fern stem) in which the sieve plates in the phloem cells are most delicately preserved—by far the finest example of preservation of phloem tissue that I know of. Fungus mycelium is occasionally found in coal-ball plants, although it is not common, probably because of the anaerobic conditions under which most of the plant remains were quickly engulfed. An exceptional case was reported a few years ago (9) of a fern rhizome containing very abundant mycelium in the cortex. It is not only preserved with surpassing excellence but also presents morphological characters which suggest that it functioned as a mycorrhizal association.

HISTORICAL

Since no comprehensive review of American coal-ball studies has been given recently it seems appropriate to record here what is

known of the origins of this still rather young phase of botanical-geological science. The earlier references cited below have been brought to my attention by colleagues and do not represent a thorough combing of the literature.

The earliest published American record of these petrifactions is given by C. R. Keyes (57, pp. 279–281) in the second volume of the Iowa Geological Survey Reports. To quote directly from this account: "Associated with the coal bed are numerous lenticular masses of ironstones. They vary in size from a few inches to ten or twelve feet horizontally, and up to six feet vertically. The smaller ones are commonly called 'nigger heads'. These aggregations occur in all parts of the coal seams, but they are most abundant in the roof and upper surfaces of the coal, where they are often so close together as to form an almost continuous layer. It is common for the nodules in the upper part of the coal to project upwards above the top of the seam, often two to three feet in case of the larger masses. They are covered usually by a few inches of carbonaceous material".

In the Annual Report for 1896 A. F. Bain (14, p. 297) reported the presence of "Clay-ironstones" in the Bloomfield mine and several others, and he notes that: "An analysis of similar material from a mine in Mahaska County showed the presence of about 88 percent of limestone, with 8 percent of organic matter and small percentages of iron oxides and sulphides".

The authors in both instances figure vertical sections through the coal, showing the coal balls ("ironstones") in the upper portion of the seams. Thus there is no possibility of their being confused with the sterile slate-gray concretions common in the overlying shales and referred to by the miners as "nigger heads". The presence of eight per cent organic matter removes any doubt as to their identity, and the high percentage of limestone reported by Bain also suggests coal balls of fine quality. At least his figures leave little room for the obnoxious iron sulphide.

The coal balls in these reports are not referred to as such, and aside from the fact that it was recognized that they contained organic matter no mention is made of fossil plants. If they were known to paleobotanists of the day apparently no studies of them were undertaken.

A few years later W. S. Gresley (38–40) presented, in a series

of three papers in the American Geologist, descriptions of plant structures which he observed in coal. The third number of the series deals with plants found at What Cheer, Iowa, and it is clear that these were in coal balls: "The pyritous concretions in the What Cheer coal furnish us with samples of what may be called the *raw material* of coal saved from coalification by these concentrations of iron, sulphur, lime, etc.".

Gresley described *Cordaianthus* fructifications, cordaite seeds and *Pecopteris* foliage from the What Cheer coal balls, and it is of interest to note that these were brought to the attention of David White. "In these pyrites concretions in the coal bed at What Cheer in Iowa, this species of *Pecopteris* is rather common. It was by grinding and polishing that the structural details were brought to light, and I am greatly indebted to Dr. David White of the United States Geological Survey for suggesting that this fossil be figured, because probably of scientific value paleobotanically. Instances of fossilized anatomical structure in the leaves of ferns are certainly very rare, in fact this is about the only case I can find or know of". (p. 13)

Although Gresley was familiar in a general way with the work of Williamson, Carruthers, Dawson, and Seward, he was not a botanist and made no pretense of presenting thorough paleobotanical descriptions. It seems significant to note that Gresley's account appeared during the peak of coal-ball studies in England. Williamson's "Organization" series (101) was completed as well as Williamson and Scott's "Further observations" (102), and Scott's own prolific output was well under way, yet no efforts seem to have been made to discover comparable petrifactions in the Upper Carboniferous coals of America.

In spite of these earlier records and the work of the British paleobotanists, it was not until the early 1920's that coal balls were recognized as such in this country. In 1921 G. H. Cady of the Illinois Geological Survey discovered them in a mine in Harrisburg, Illinois, and these were turned over to A. C. Noé. In 1925 Noé wrote: "The existence in America of coal balls was suspected by the author who succeeded in obtaining in 1922 the first specimens of genuine American coal balls. These came from Harrisburg, Illinois; later good specimens were found in Danville, Illinois, in Calhoun (Richland County), Illinois, and in Streator, Illinois. Other American deposits are in Iowa where coal balls

showed up at Des Moines, Indianola, and Chariton. Good specimens were also collected by the author near Sturgis, Kentucky, and at Cayuga, Indiana". In another paper Noé (67) reported that he had received coal balls from Newcastle, Texas.

In view of the fact that Professor Noé is responsible for the actual initiation of American coal-ball studies it seems fitting to include in this review a few lines on what was in certain respects a colorful career. Born in Gratz, Austria, in 1873, he led a rather secluded life until the age of fourteen when, for the first time, he attended school with other boys. Following graduation from the Gymnasium he served his two years of military service and later entered the University of Gratz where he had opportunity of studying under Constantin von Ettingshausen, one of the distinguished paleobotanists of the time. After Gratz his studies were continued for two years at the University of Göttingen, and then he came to America. Here he served first as an instructor of German at Stanford University and later in the same capacity at the University of Chicago. In the early 1920's he was appointed to a Professorship in Paleobotany and given opportunity to explore for coal balls which he apparently had a strong conviction must occur in the American coals. His initial success in this direction led to his appointment to the summer staff of the Illinois Geological Survey under whose auspices much of his exploration of the Illinois coals was carried on. Although his actual scientific contributions were few, it is remarkable how vivid his memory remains with the mine operators and workers throughout the area which he explored. His ability to meet on equal terms the top executives in the industry or the miners underground facilitated in no small degree his rather extensive collecting activities.

A consideration of collecting activities in this period would not be complete without mentioning the work of Frederick O. Thompson of Des Moines, Iowa. Although not a professional paleobotanist, Mr. Thompson has contributed greatly to our knowledge of the coal-ball floras through his extensive field work. In about 1932 he started collecting nodules at the famous Mazon Creek locality north of Coal City, Illinois. Most of that material was sent to the Harvard Botanical Museum, and a few years later (1938) he began a search for coal balls, chiefly in Iowa and eastern Kansas. Much of this also was sent to Harvard, although generous portions were turned over to the present writer. I may say

that I have never known a collector who so generously gave his material, with so little thought of personal recognition, to those who he considered might be able to put it to the best use. So far as I am aware Mr. Thompson's collections were far greater than those that have been made by any other worker.

The bulk of American coal-ball collections have been made thus far in the southern parts of Illinois, Indiana and Iowa, and eastern Kansas. They are also reported from Kentucky, Texas and Missouri, and from personal communications I have been informed that they have been found in Ohio, West Virginia and Oklahoma, but our knowledge of their occurrence and distribution in these last three States is still very sketchy. Even in southern Illinois and south central Iowa, where most of the collecting has been done, there remains much exploratory field work for the future.

COAL-BALL HORIZONS

A consideration of the stratigraphic distribution of American coal-ball horizons and of their correlation with European horizons has been given by Schopf (92). Only a brief résumé will be included here together with notations on a few new discoveries made since the time of his publication.

As may be noted on the accompanying chart, seven coal-ball horizons are known for the Illinois-Indiana area. Those from the Shumway are unique in that they are silicified. The Calhoun horizon was early exploited by Noé and has produced some of the finest preserved plants found in this country. Many of these specimens compare very favorably with the best of the English material.

A considerable quantity of coal balls has been obtained from the Herrin (No. 6) coal. These were first reported at Nashville, Illinois, by Cady in 1936; we have also found them in some abundance at the Pyramid mine south of Pinckneyville. The Rock Island (No. 1) coal in the upper part of the Tradewater group is the lowest horizon at which coal balls have been located. They have also been reported in Illinois from the Danville (No. 7) and the Harrisburg (No. 5) coal. It was in fact at the latter horizon at which coal balls (recognized as such) were first found in America.

In Indiana the Parker and Petersburg V coals have produced

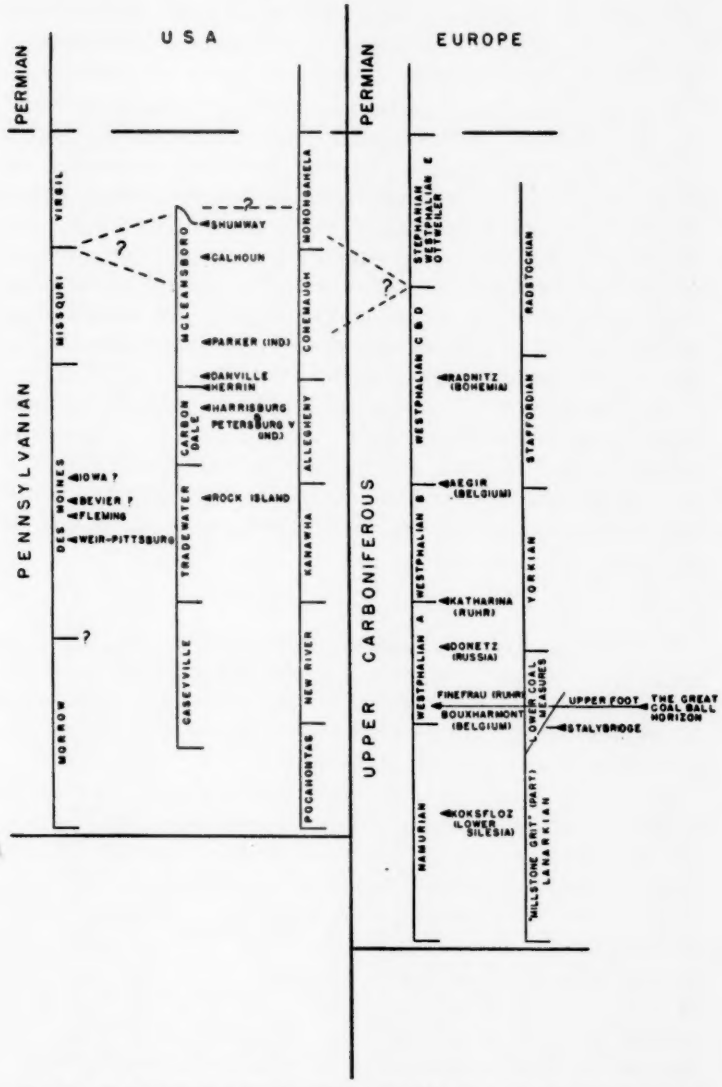


FIG. 1. Chart showing stratigraphical position of American and European coal-ball horizons. (*From Schopf* (92), *modified*).

petrifactions. Explorations have only begun in the southern part of that State, and it seems very likely that more localities will turn up in the next few years.

During the past year we have obtained considerable quantities of coal balls from the Fleming coal a few miles south of Mineral, Kansas. The Fleming is a coal of variable thickness and occurrence which lies ten to 20 feet above the Mineral coal and occurs toward the upper part of the Cherokee shale (1). On a more recent collecting trip in the southeastern Kansas area we have located another deposit of coal balls which apparently were taken from the overlying Bevier coal. Darrah (30) has reported coal balls from the Weir-Pittsburg coal which lies below the Fleming horizon and near the middle of the Cherokee shale.

Mr. Frederick O. Thompson and others have collected coal balls from numerous localities in Iowa. Darrah (30) has reported 13 mines in that State, and, judging from the significant plants that have been found thus far, it will continue to be a fertile field. Adequate publications are not as yet available on the stratigraphy of that area.

THE FOSSIL PLANTS

None of the coal-ball localities studied to date can be said to have been thoroughly investigated, although in a few cases we have a rather clear picture of the dominant elements. This is not a reflection on the workers in this field but rather indicates the abundance of material that awaits study.

Just what constitutes a thorough investigation of a coal-ball locality is not easy to define. The dominant plants will be scattered in abundance through most of the petrifications, but the rarer specimens and particularly the smaller ones are encountered partly by chance. After examining a few hundred coal balls from a particular locality a fairly clear picture of the dominant species will be acquired. It may be necessary to collect much larger quantities in order to select especially well-preserved specimens and obtain as complete a picture of an individual species as possible. But regardless of how much collecting is done there is always the possibility that the next specimen will contain something new, and unless each coal ball is cut into rather thin slices certain of the smaller plants may be lost. We are presently investigating a

beautifully preserved cone in my laboratory which measures only a little over 2.5 mm. in diameter. There is obviously a rather large element of chance involved in the recovery of such " microfossils ".

As an introduction to a consideration of some of the more important fossils I have compiled in the following chart a list of the plants reported in American coal balls. There may be a few omissions, although I believe that it is fairly complete. In some cases species have been reported by name only, while in others the description varies from being sketchy to quite complete. Our knowledge of the fossils varies accordingly, and, while there is not a plant on the list that we do not want to know more about, certain recent studies are extremely encouraging with regard to what may be expected in another few decades.

The Fossil Plants Reported From American Coal Balls

FUNGI

Mycorrhizae, in fern rhizome (9)

COENOPTERIDALES

Anachoropteris clavata Graham (30, 33, 36)
A. decaisnii Renault (33)
A. involuta Hoskins (28, 30, 33, 46)
A. pulchra Corda (33)
Ankyropteris grayi (Williamson) P. Bertrand (28, 30)
A. westphaliensis P. Bertrand (33)
Botryopteris americana Graham (28, 30, 33, 36)
B. cf. *cylindrica* Williamson (33)
B. forensis Renault (33, 36, 46)
B. fraiponti Leclercq (?) (30)
B. globosa Darrah (25, 30)
B. hirsuta (Williamson) (30, 33, 46)
B. radiata Darrah (28)
B. cf. *ramosa* Williamson (33, 36)
B. renaulti C. E. Bertrand and Cornaille (28)
B. sp. (18)
Etapteris scotti P. Bertrand (28, 30, 33)
E. lacattei P. Bertrand (33)
Notoschizaea robusta Graham (33, 35)
Scleropteris illinoiensis Andrews (2)
Stauropteris americana Darrah (28)
S. sp. (30)

LEPIDODENDRALES

Bothrodendron mundum Williamson (30, 33)
B. sp. (77)
Illinoicarpon cadyi Schopf (87)
Lepidocarpon glabrum Darrah (6, 18, 27)
L. ioense Hoskins and Cross (50)
L. lomaxi Scott (26, 30, 33, 59, 81)
L. magnificum Andrews and Pannell (11)
Lepidodendrid megaspores (18)
Lepidodendrid microspores (18)
Lepidodendron harcourtii Witham (70)
L. scleroticum Pannell (75)
L. selaginoides Sternberg (18, 30, 33, 36)
L. sp. (45, 46)
Lepidodendron leaves (18, 33)
Lepidophyllum aciculum Reed (81)
L. alatum Reed (81)
L. latifolium Graham (37)
L. thomasi Graham (37)
L. trichosulcata Reed (81)
Lepidostrobus axis (81)
Lepidostrobus cone scale (81)
Lepidostrobus megaspores (81)
Lepidostrobus sp. (18, 33, 36)
Mazocarpon oedipternum Schopf (93)
M. shorense Benson (33)
Miadesmia sp. (30)

Sigillaria approximata Fontaine and White (30, 33, 36)
S. elegans Kidston (33)
Sigillariopsis cordata Reed (81)
Stigmaria ficoides Brongniart (33, 45, 81)
Stigmaria, rootlets (18, 30)

EQUISETALES

Elaterites triferens Wilson (105)

CALAMITALES

Annularia sp. (33, 45)
Asterophyllites multifolia Reed (79)
Calamites communis (Binney) (30, 33, 77)
C. multifolia Reed (79)
C. cf. *suckowi* Artis (30)
C. sp. (18, 36)
Calamostachys sp. (18, 36)
Macrostachya thompsonii Darrah (30)
M. sp. (24)

SPHENOPHYLLALES

Bowmanites trisporangiatus Hoskins and Cross (52)
Sphenophyllum dawsoni Williamson (30)
S. plurifoliatum Williamson and Scott (16, 18, 30, 33, 36, 77, 84)
Sphenostrobus thompsonii Levittan and Barghoorn (62)

MARATTIALES

Asterotheca sp. (18)
Cyathotrachus bulbaceus Graham (33, 35)
Myriotheca sp. (18)
Psaronius illinoensis Hoskins (33, 49)
P. sp. (18, 30, 33, 36, 45, 66, 84)
Ptychocarpus unita (Brongniart) (48)
P. sp. (18, 70)
Scolecopteris latifolia Graham (33, 35)
S. minor Hoskins (33, 35, 43)
Stipitopteris americana Lenz (61)
S. sp. (18).

PTERIDOSPERMEAE

Conostoma oblongum Williamson (56, 59, 80)
C. platyspermum Graham (33, 35)
C. quadratum Graham (33, 35)
Dolerotheca formosa Schopf (93)
D. reedana Schopf (93)
D. schopfi Baxter (17)
D. sclerotica Baxter (17)
D. villosa Schopf (93)
Heterangium americanum Andrews (3)
H. grievei Williamson (33)
H. tiliaeoides Williamson (33, 36)
Lagenostoma ovoides Williamson (30)
Lyginopteris oldhamia (Binney) ? (30)
L. sp. (33, 77)
Medullosa anglica Scott var. *thiesseni* Schopf (4, 90)
M. anglica Scott var. *ioensis* Andrews and Kernen (8)
M. distelica Schopf (90, 91)
M. elongata Baxter (17)
M. endocentrica Baxter (17)
Medullosa noei Steidtmann (17, 30, 35, 88, 98)
M. primaeva Baxter (17)
M. thompsonii Andrews (4)
M. sp. (4, 33)
Medullosa, roots (47)
Myeloxylon americanum (Hoskins) Schopf (10, 42, 73)
M. bendixenii Andrews (4)
M. sp. (18, 33)
Microspermopteris aphyllum Baxter (17)
Physostoma sp. (30)
Schopfiastrum decussatum Andrews (4)
Sphaerostoma ovale Williamson (33)
Stephanospermum akenoides Brongniart (59)
Telangium pygmaeum Graham (33, 35)
T. sp. (24)

CORDAITALES

Amyelon radicans Renault (30, 33)
Cardiocarpon affinis (Lesquereux) Brongniart (82)
Cardiocarpus spinatus Graham (33, 36)
Cordaianthus shuleri Darrah (26, 30)
Cordaicarpus spinatus Graham (26, 30)
C. sp. (18, 26, 30)
Cordaites crassus Renault (30)
C. felicis (30)
C. iowensis Wilson and Johnston (104)
C. sp., small leaves (30)
Kamaraspermum leeanum Kern (67)
Mesoxylon nauertianum Andrews (2)
M. poroxyloides Scott and Maslen (30)

Mitrospermum compressum (Williamson) Arber (30)
Nucellangium glabrum (Darrah) Andrews (6)
Pachytesta gigantea Brongniart (30, 80)
P. noei Hoskins and Cross (54)
P. vera Hoskins and Cross (53)
Rabdospermum spinatum Andrews (56)
Rotodontiospermum illinoense Arnold and Steidtmann (13, 87)
Trigonocarpon parkinsoni Brongniart (59)
T. pusillus Brongniart (59)
Trigonocarpus sp. (30, 70)

FERN—PTERIDOSPERM FORM GENERA

Alethopteris serlii Brongniart (30)
Linopteris munsteri Eichwald (30)
L. cf. *obliqua* (Bunbury) (24)
Neuropteris ovata Hoffman (30)
N. rarinervis Bunbury (18, 24, 30)
N. scheuchzeri Hoffman (24, 30)
Odontopteris cf. *genuina* Grand'Eury (30)
Pecopteris arborescens Schlotheim (30)
P. dentata Brongniart (30)
P. miltoni Artis (30)
Sphenopteris cf. *neuropteroides* Boulay (30)

LYCOPODS

The arborescent lycopods constituted one of the abundant elements in the Pennsylvanian forests of the central States. The remains of stems, leaves and cones are frequently encountered, although in variable percentages with reference to the associated fossils. At some localities the lycopods are relatively scarce and at others their abundance is indicative of their dominance in the flora.

Coal balls from the Pyramid mine (Herrin coal) two miles south of Pinckneyville, Illinois, contain about 70% stem remains of *Lepidodendron scleroticum*. This is a seemingly unique species characterized by an abundance of sclerotic (stone cell) nests in the cortex. Fragments of large stems, branches and small twigs have been described, showing an interesting transition in the stelar structure which follows the size and form principle of Bower. The smallest twigs have a minute primary body of less than one mm. diameter, protostelic or nearly so, and medullation increases with the larger specimens which have a siphonostele of more than eight mm. diameter. Fragmentary specimens of much larger steles have been found with abundant secondary wood.

The European *Lepidodendron selaginoides* has been reported by a number of investigators, but while it seems altogether likely that the identifications are correct, no comprehensive studies of this species have been made. Indeed the only American arborescent lycopods that have been dealt with at all critically are *L. johnsonii* Arnold from the lower Pennsylvanian of Colorado (not a coal-ball plant) and *L. scleroticum*.

Within recent months we have obtained large quantities of *Lepidodendron* and a few *Lepidophloios* stems from eastern Kansas. The steles alone in some of these specimens exceed six cm. in diameter, and it is interesting to note that there is considerable variation in the ratio of the diameter of the primary body, and the thickness of the primary and secondary wood. A study of this material will undoubtedly add considerably to our knowledge of these plants.

The diversity present in the aborescent lycopods is also attested by the numerous species of leaves that have been described by Reed, Graham, and by Wilson and Tillapaugh. The latter authors have recognized the necessity of serial sections through more or less complete leaves in order to eliminate a multiplication of names based on different parts of a single leaf. Certain of these lycopod leaves are of particular interest by virtue of their "xerophytic" anatomy, the epidermis and multi-layered hypodermis having heavily thickened walls suggestive of an extreme adaptation for the prevention of water loss.

It seems evident from studies of their reproductive structures that these arborescent forms (*Lepidodendron* and *Lepidophloios*) represent the peak of evolution in the lycopod group as a whole. Numerous seeds assigned to *Lepidocarpon* have been described. It is my own opinion that most or all of the American Pennsylvanian lepidodendrons bore seeds of the *Lepidocarpon* type. The more primitive bisporangiate cones are conspicuous by their absence from American coal balls. The bisporangiate cone *Lepidostrobus noei* Mathews (65), believed to be from the upper Devonian of Kentucky, indicates their existence in the region in former times, but it is clear that *Lepidocarpon* had gained dominance in the Pennsylvanian.

The English *Lepidocarpon lomaxi* has been reported by several workers, and among the better known American species may be listed *L. ioense* and *L. magnificum*. In the latter, microspores were found within the seeds which are identical with those contained in an associated microsporangiate cone.

An especially significant step in our understanding of the lycopods was made by Schopf (92) in his paper dealing with *Mazocarpon oedipternum* from the Calhoun horizon in southern Illinois. *Mazocarpon* is represented by strobili in which the megaspores

(eight per sporangium in *M. oedipternum*) are embedded in a massive parenchymatous tissue. The megaspores and microspores are borne in separate cones. In some specimens the gametophyte contained within each megaspore is well preserved and displays a single large archegonium. Otherwise they are quite similar to the gametophyte of *Selaginella* and *Isoetes*. Schopf has shown that these cones correlate closely if not congenerically with the compression fossils known as *Sigillariostrobus*. Furthermore, studies of the comparative anatomy of the *Mazocarpon* stelar system and the peduncles found in *Sigillaria* stems (36) leave little doubt that the latter bore the *Mazocarpon* cones.

It is perhaps worth noting that, while the stem anatomy of *Sigillaria* compares closely with that of *Lepidodendron*, the two genera are strikingly different in both the organization of their cones and the way in which they were borne. In the former they were produced along the trunk or large branches, while in *Lepidodendron* they were apparently always terminal.

ARTICULATES

A number of significant contributions have been made in recent years dealing with the vegetative organs and supposed cones of the Sphenophyllales. *Sphenophyllum* stems are commonly encountered in many of the coal-ball localities, sufficiently so as to indicate beyond doubt that it was one of the most ubiquitous elements of the vegetation throughout Pennsylvanian times.

The stems are often well preserved, and Baxter (16) has made a critical study of numerous specimens from an Illinois and an Iowa mine. He reports a considerable degree of variation in the anatomy of the stem and, although recognizing three types of structures, places his specimens in *S. plurifoliatum*. In view of the diversity of the foliage found as compressions, as well as that of the structure of cones assigned to the group, it appears probable that future studies will segregate the stem specimens into a number of species.

Recent contributions to our knowledge of sphenophyll cones are particularly noteworthy. In 1943 Hoskins and Cross presented the results of their monographic treatment of the genus *Bowmanites* in which they included a detailed description of a new species from Iowa. *Bowmanites* includes fructifications that are referable

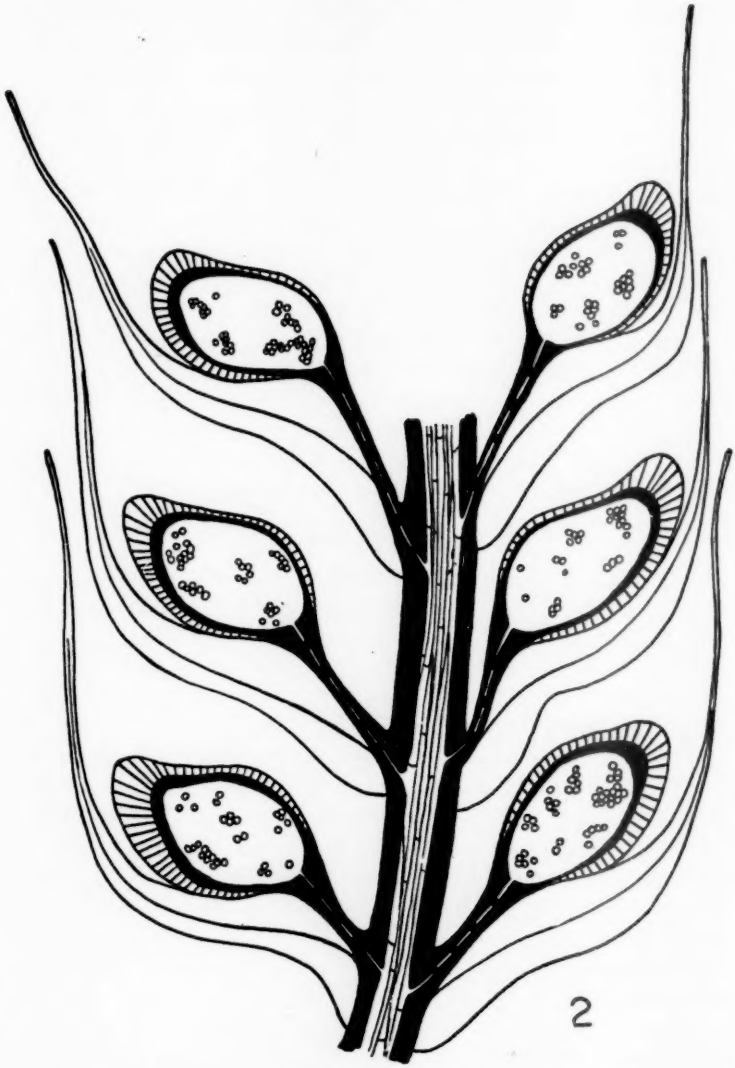


FIG. 2. A diagrammatic median longitudinal section of *Sphenostrobus thompsonii*. (*From Levittan and Barghoorn* (62)).

to *Sphenophyllum*. The cones consist of whorls of sterile bracts each of which bears one or more sporangia on its upper surface; where more than one sporangium is present the sporangiophores are of unequal length. *Bowmanites trisporangiatus* from Iowa is a beautifully preserved cone 1.6 cm. in diameter and in excess of six cm. long. Three sporangia are borne on each of the 18 sporophylls composing a whorl. Although the genus is characterized by Hoskins and Cross as being homosporous, in a more recent paper Arnold (12) gives reasons for including heterosporous forms as well. He has described such a specimen (*Bowmanites delectus*) from the Michigan coal basin (not a coal-ball plant) consisting of cones about 1.7 cm. in diameter. It is composed of 60 or more closely packed whorls with the sporangia borne in pairs. Both micro- and megasporangia are borne in the same whorl and are similar except for the spore size, the microspores being 75–90 μ in diameter and the megaspores 660–750 μ in diameter.*

We have recently discovered a very small and well-preserved cone measuring only three mm. in diameter in an Illinois coal ball that is apparently referable to *Bowmanites*.

Another unique cone which also may be of sphenophyllaceous affinities has been described from an Iowa coal ball by Levittan and Barghoorn (62). Assigned to a new genus, *Sphenostrobus*, this cone is distinctive in its tetrarch protostele and sessile sporangia. The entire cone measures about nine mm. in diameter and is composed of 16 sporophylls in each whorl. The sporangia, also 16 in a whorl, are sessile, being attached by their inner ends and alternate with the sporophylls. The four-parted arrangement of the stele and strobilar appendages as well as the sessile mode of attachment of the sporangia place this cone in a unique category. Although it presents points of similarity with certain species of *Bowmanites*, the authors have also considered possible affinities with the Noeggerathiales.

Remains of the arborescent articulates, the calamites, have been notably scarce in American coal balls to date. Stems have been reported from Illinois and Iowa but most of the specimens are small and poorly preserved. Since *Annularia* and *Asterophyllites*

* More recently this has been transferred to the genus *Discinites*; see Arnold, 1949, Contrib. Mus. Paleont., Univ. Mich., vol. 7, p. 220.

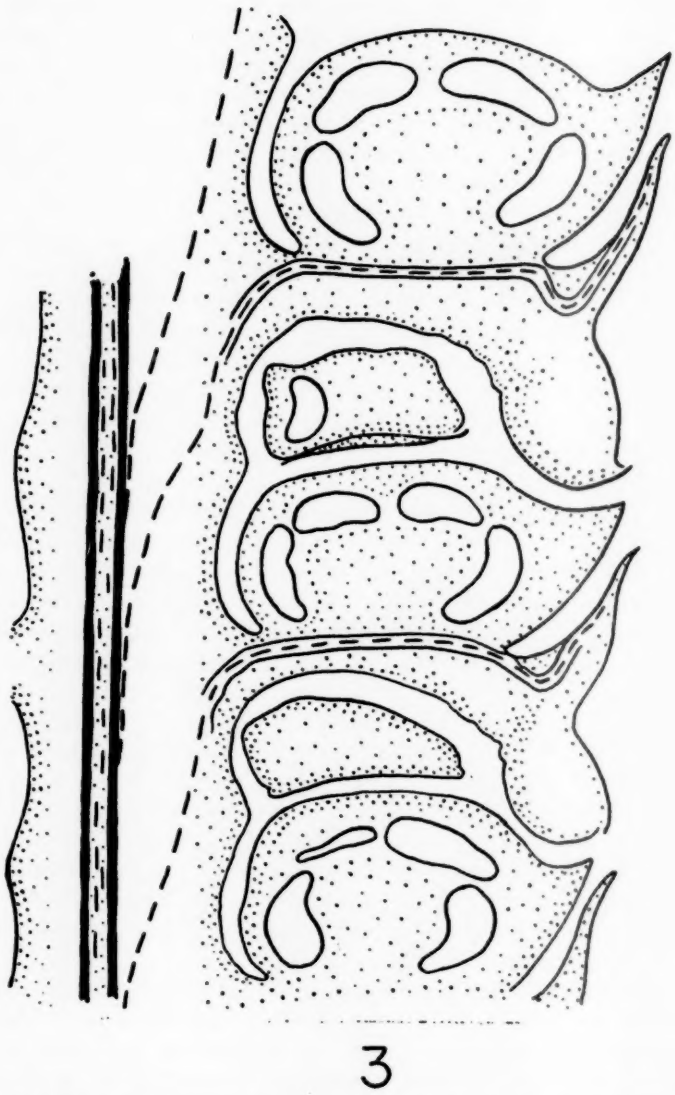


FIG. 3. A diagrammatic longitudinal section of *Mazocarpon oedipternum*. (*From Schopf* (92)).

foliage is not uncommon in compression form in the American Carboniferous it seems rather odd that calamite stems have not been found more often. Recent collections from the Fleming coal of Kansas, however, contain numerous calamite specimens representing stems eight to ten inches in diameter. A few have also been found in the Calhoun horizon in Illinois.

Fredda Reed has described a small calamite stem (*Calamites multifolia*) from the Harrisburg, Illinois, locality. Associated with this were *Asterophyllites* leaves, of interest because of the conspicuous thick-walled fibrous bundle sheath composing a large portion of the leaf. Isolated sporangiophores were described in the same contribution which bear lobed peltate heads each with 25–30 sporangia. Continued investigations of such fructifications should help to bridge the gap between the relatively simple Devonian forms, such as *Hyenia* and *Calamophyton*, and the known calamite cones of the Carboniferous.

The discovery of elater-bearing spores in an Iowa coal ball (103) presents a notable addition to our knowledge of Carboniferous articulates. These spores (*Elaterites triferens*) average about 70 microns in diameter, including the perispore of coiled elaters, and are thus about twice the size of the spores of modern *Equisetum*. They also differ from a living horsetail in that the manner of coiling of the elaters is somewhat different and they are three in number instead of four.

Our knowledge of Paleozoic articulates that appear to be closely related to the modern family is very scanty. Stems and cones assigned to *Equisetites* have been reported from the Upper Carboniferous of England. Wilson's *Elaterites triferens* offers an additional tantalizing clue, and it is to be hoped that further researches will reveal the remainder of the plant.

COENOPTERIDALES

Among the more interesting fossil plants of the European and American coal-ball floras is a rather indefinitely defined assemblage of primitive "ferns" known as the coenopterids (Coenopteridales of Bower). Relatively few of them are known with any degree of completeness, but in general they are characterized by protostelic stems, terminally borne sporangia, "fronds" which branched in three dimensions and in most cases (if not all) lacked

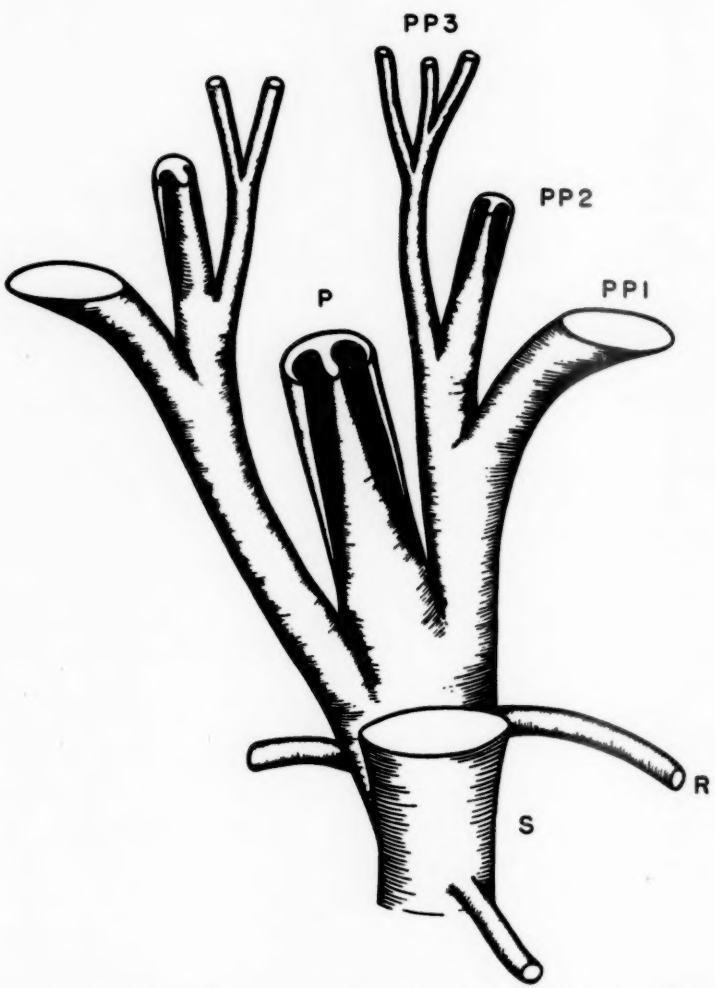


FIG. 4. The vascular system of the basal part of a frond of a new species of *Botryopteris*. S, stem stele; P, central strand resulting from first trisection of trace; PP1, proximal secondary pinna trace; PP2, median secondary pinna trace; PP3, distal secondary pinna trace; R, root.

a distinct lamina. Some were small plants which very likely were epiphytes. The group is of particular interest for a number of reasons; it is an assemblage of plants that is clearly more advanced than the psilophytes of Pre-Carboniferous times yet is generally more primitive than the fern families (Marattiaceae, Osmundaceae, Schizaeaceae, Gleicheniaceae) that appear to have become well established in upper Paleozoic times.

The coenopterids present a rather diverse assemblage which should not be confined by the usual mental reservations of familial or ordinal boundaries. Judging from what is known of them at present it seems possible that they represent an evolutionary advancement of the earlier psilophytes and may in turn have produced certain of the above-mentioned fern families as well as the pteridosperms. If the coenopterids that we now know are not the actual ancestors of the ferns and seed-ferns they at least present morphological and anatomical phases through which the latter almost certainly passed.

Remains of the coenopterid ferns, particularly the genus *Botryopteris,* are of common occurrence in coal balls from many localities. Nine species of *Botryopteris* have been reported, some of which have been assigned to European species, while others are considered to be new. Some of these have been based on rather small portions of the plant and it is not possible to know whether they all represent distinct species. It may not be amiss here to describe in some detail a specimen (10) recently found in southern Illinois which throws considerable light on the morphology and taxonomy of *Botryopteris* as well as the evolution of the macrophyllous leaf (Fig. 4). (See Addenda and 64b.)

The specimen was found in a small coal ball about 15 cm. long by eight cm. in diameter and consists of a stem with parts of five fronds attached and numerous smaller portions associated. Of special interest is the anatomy of the frond. The " trace " at the point of its departure from the protostele of the stem is at first tangentially elongated and somewhat larger than the stele itself. This departing segment soon divides into three portions, the central one assuming the characteristic tridentate form in transverse section. The two lateral strands (primary pinna traces) remain terete, resembling the stem stele. Each of these primary pinna traces next trisects, and at right angles to the first division. Of

this second division in each case the central strand assumes the botryopterid W form while the laterals remain terete. The proximal one is the larger of the two and identical with the stem stele except that it is slightly smaller. These lateral strands then divide a number of times, as shown in the accompanying figure.

There is no evidence here or in any of the other botryopterids that the frond was laminated. The smallest divisions are small round branches which show no evidence of flattening. The significant features of these organs are that in their anatomy they present a medley of foliar and stelar characters, the branching is three-dimensional, and a lamina is apparently lacking. They are clearly unit appendages of the stem so that it seems to be entirely valid to consider them as primitive fronds or structures that are more or less intermediate between a rhyniaceous branch system and a true fern frond.

Unfortunately the above-described specimen is sterile. In 1939, however, Darrah (25) described a remarkably well-preserved fructification under the name *Botryopteris globosa.* It consists of a huge aggregation (about $5 \times 5 \times 6$ cm.) of sporangia, each of which measures 1.5×1.0 mm. It is a surprisingly large fructification in view of the fact that *Botryopteris* plants apparently were rather minute. It is probable that the secondary pinna described above was a pendant structure, the branches of which may have borne such clusters of sporangia.

Darrah (28) has described a new species of *Stauropteris* and has reported *Etapteris scotti* from Iowa coal balls. A few years ago Sahni (84) showed a species of *Etapteris* to be borne on a stem of the *Botrychioxylon* type. The latter, previously known from the British coal fields, is unique in that it is a fern stem with well-developed secondary wood. Our present knowledge of *Etapteris* from American coal balls is still scanty. I think it is likely that the plant described a few years ago as *Scleropteris illinoiensis* (2) actually belongs in *Botrychioxlyon.* We have recently encountered additional specimens in the Kansas coal fields, and there is a strong likelihood that it will be possible to contribute more to our knowledge of this significant coenopterid.

Ankyropteris grayi, another particularly interesting coenopterid, has also been briefly described from Iowa coal balls (28). We have recently found a fine specimen in excess of ten inches long

in an Indiana petrifaction which should contribute appreciably to our understanding of this plant. It may be in order to mention briefly another species, *Ankyropteris hendricksi,* described by Read (76) from the early Pennsylvanian Johns Valley shale of Oklahoma (not a coal-ball plant). It consists of a stem with numerous large petioles, with their characteristic H-shaped vascular strands, bound together in a dense mass of epidermal hairs and adventitious roots, the whole approaching in size that of a small tree fern.

Thus far American coal-ball studies have brought to light but little in the way of coenopterid fructifications. Graham (35) has described a new genus (*Notoschizaea*) which bears a resemblance to the European *Corynepteris* Zeiller. Beyond this similarity, however, the affinities of *Notoschizaea* must remain problematical, pending further discoveries. Graham (37) also reported fructifications in association with his specimens of *Botryopteris americana.*

In a recently completed but yet unpublished paper, Mamay has recognized three additional fructification species of *Botryopteris.* These are particularly interesting from the standpoint of the great variability exhibited by the spores. Furthermore, the delicate annulate nature of the sporangial walls, along with the comparatively low spore output in at least one species are suggested by Mamay as indicating a true leptosporangiate condition in this genus, notwithstanding its anatomical primitiveness. (See 64*a*.)

MARATTIACEAE

It is evident that the Marattiaceae, represented by trunks and fertile leaf specimens, constituted one of the abundant floral elements in the American Upper Carboniferous. Very little of this material has been studied, however, to date. The fossil remains found in the coal balls that are assigned to the family consist of petrified stems referred to *Psaronius* and synangial fructifications which are generally similar to certain of the modern genera.

The genus *Psaronius* includes tree fern trunks, some of which attained considerable magnitude. The vascular system in many of them is a highly complex polycyclic dictyostele. A great many species are known from Europe but so far as I am aware only one (*Psaronius illinoensis,* 49) has yet been described from American coal balls. A few have been described from other sources in this country, of which Gillette's contribution (34) is one of the

more recent. His paper cites much of the significant earlier literature.

Certain of the Illinois coal-ball deposits produce huge quantities of *Psaronius* roots. They are rather large organs, a centimeter or two in diameter and characterized by a small star-shaped stele with a very broad aerenchymatous cortex. Masses of hundreds of pounds of these roots are not uncommon.

Recently considerable numbers of well-preserved *Psaronius* trunks have been found in southern Illinois and they are especially abundant at the Mineral, Kansas, locality. They display considerable diversity in stelar structure, in all probability representing a rather large number of species. Studies of these fossils which are now in progress will contribute notably to a better understanding of the arborescent constituents of the Carboniferous forests.

It is thought that certain of the large pecopterid fronds were borne by the psaronii, and rather fine reconstructions have recently been given in a paper by Corsin (20).

Of the supposed marattiaceous fructifications found in our coal balls *Scolecopteris* species are the most frequently encountered. Graham (35) and Hoskins (43) have devoted some attention to the genus, and in a recently completed study Mamay has recognized four new species. *Scolecopteris* shows certain points of resemblance to the coenopterid genus *Chorionopteris,* and there is reason to suppose that the former may be a derivative of *Chorionopteris.*

Cyathotrachus has also been reported by Graham (35). This genus is of interest because of its resemblance to the living *Christensenia.* A considerable number of other new marattiaceous spore-bearing organs will be described in a forthcoming paper by Mamay.

Judging from the diversity of sterile and fertile foliar organs which in all probability were borne on the *Psaronius* trunks, it appears that we are faced with a problem that is comparable to the taxonomic treatment of the pteridosperm stem genus *Medullosa.* Many species are known for the two genera, and considerable diversity of stelar anatomy is present in both. Yet it has not been possible to draw reasonable dividing lines that would segregate each into a number of different genera. In view of the im-

probability of ever finding many of the fronds and reproductive organs in organic connection with the stems, it is likely that both genera will continue to accumulate many more species for some time to come.

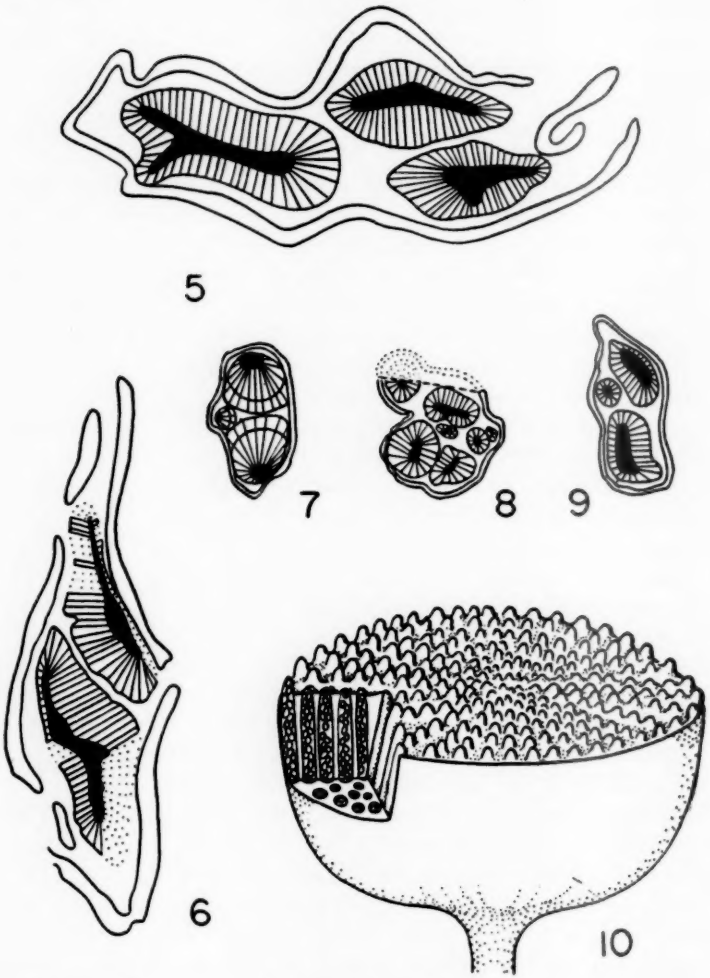
PTERIDOSPERMS

The most significant information that the American coal balls have yielded with regard to the seed-ferns is an abundance of medullosas and the associated leaves, seeds and male organs which they probably bore. Eight species and varieties of *Medullosa* are given in the accompanying list, and recent collections include at least two more. These stems possess a fibrous outer cortex, typical of the pteridosperms in general, and a polystelic vascular system with from two to a dozen or more steles. The petioles are unique in the scattered distribution of many small bundles, which lends a superficial appearance to that of a monocot stem.

Medullosa anglica may best serve as a starting point in considering the American medullosas, since it has been so widely referred to in the literature. Two varieties of this species have been reported, *M. anglica* var. *thiesseni* and *M. anglica* var. *ioensis*. These, as well as certain undescribed specimens in the writer's laboratory, indicate that the English species or very closely related ones were present in the American Carboniferous forests. Some of the others are unique, being quite unlike anything reported from Europe.

Medullosa distelica (89) is one of median size measuring eight × four cm. with two asymmetric steles, their secondary wood being strongly developed toward the center of the stem. *M. endocentrica* (17) is even more asymmetric and is known only from the stelar system. It was a small plant, two of the steles measuring five × five mm. and a third one two × one and one-half mm. *M. thompsonii* (4) is a tristelar species which displays relatively much less excentricity of the cambial activity. *M. primaeva* (17), also from an Iowa coal ball, is unique in that, although it is not entirely complete, the specimen (that of a rather small stem) displays at least six steles.

Quite in contrast to these relatively small stemmed plants is *M. noei*, described (96) from the Calhoun horizon in Illinois. Although the description of *M. noei* is based on a fragment of a



Diagrammatic transverse sections of the stelar systems of several American and one English specimen of *Medullosa*. Solid black represents primary wood and radiating lines represent secondary wood.

FIG. 5, *M. anglica*; FIG. 6, *M. distelica*; FIG. 7, *M. endocentrica*; FIG. 8, *M. primaeva*; FIG. 9, *M. thompsonii*. Magnification approximately 3 ×. FIG. 10, A restoration of *Dolerotheca fertilis* (Renault) Halle. (*From Halle* (41)).

stem, it apparently represents a tristelar species attaining a diameter of about 20 cm.

We have recently found a *Medullosa* in Kansas in which the stelar system alone measures 17 × nine cm. and consists of at least 12 steles. As may be noted in the accompanying diagram, this was certainly a plant of impressive size, quite in contrast to most of the other Carboniferous medullosas.

One of the most significant contributions to paleobotany in recent decades is Halle's (41) work on the pteridosperm male fructifications. Among the fossils treated in that paper are the huge campanulate fructifications of *Dolerotheca*. They are broadly bell-shaped and consist of many long tubular sporangia immersed in a parenchymatous tissue. Three new species have recently been described by Schopf (93), based on beautifully preserved material from the Calhoun horizon, and Baxter (17) has added two more to the list.

Schopf (93) has presented strong evidence in support of the belief that the *Dolerotheca* fructifications were borne by the medullosas, and other recent studies of the group support this view.

A considerable number of seeds have now been reported from American coal balls, and, since they have been adequately discussed by Arnold in recent issues of this journal (May, 1938; July, 1948), they will be referred to only briefly here.

In view of their abundance in both species and numbers of specimens it would be of particular interest to tie up some of the isolated seeds with the medullosan stem remains. There is some evidence to believe that *Rotodontiospermum* and certain species of *Trigonocarpus* were borne by the medullosas (13, 93).

The well-known *Lyginopteris oldhamia* has been reported from American petrifactions, but conclusive evidence as to the identity of the fossils so described seems lacking to the present writer. *Heterangium* stems are not infrequently encountered. As may be noted in the preceding list, two of the English species have been reported and a third one, *Heterangium americanum* (3), which seems to be distinct from the European members of the group. *Schopfiastrum decussatum* (4) is a unique pteridosperm stem from Iowa which is characterized by a mixed protostele from which large leaf traces depart in opposite pairs, the succeeding pair alternating with the previous one. Secondary wood is present and

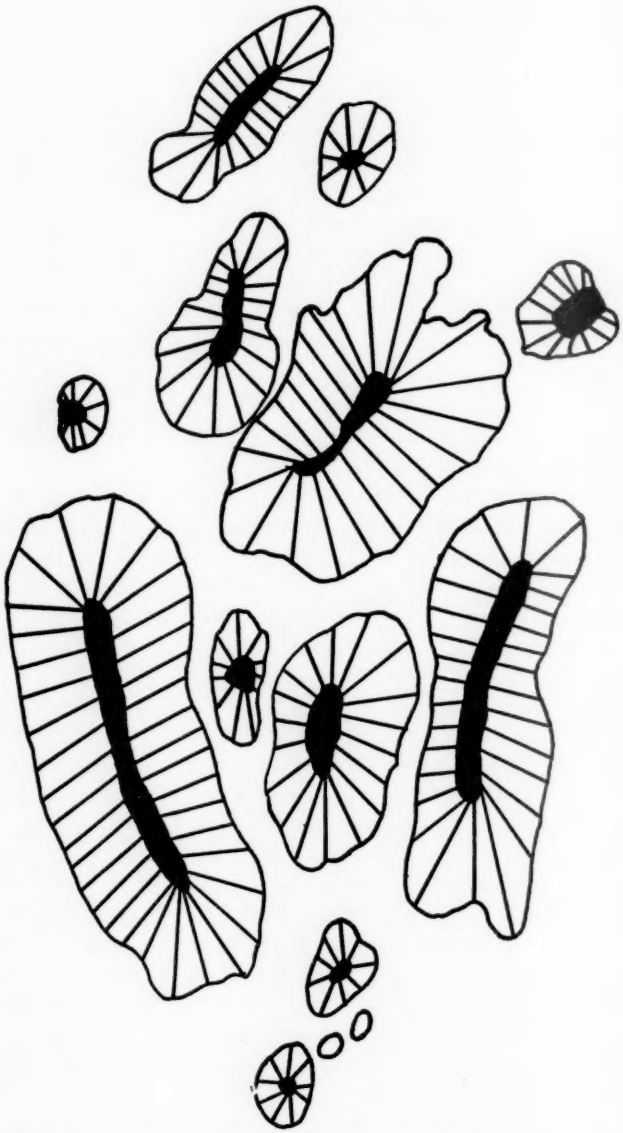


FIG. 11. Diagrammatic transverse section of the stelar system of a large *Medullosa* from Kansas. Natural size.

the outer cortex is composed of nearly parallel radial-longitudinal fibrous strands.

CORDAITALES

The stems and leaves of the cordaites are abundant in many American coal-ball localities. Several species of *Cordaites* and *Mesoxylon* have been described, although these have contributed little that was not previously reported from European material. In one species of *Mesoxylon* it has been possible (2) to establish organic connection with the roots known as *Amyelon*. No critical studies of the leaves have yet been undertaken, although they are encountered in large quantities and in some specimens the preservation is quite good.

Cordaianthus fructifications have been described from the Iowa coal balls (26). These likewise are abundant at many localities but for the most part are rather poorly preserved.

Evidence has recently been presented which suggests cordaitean affinities for the highly problematical *Nucellangium glabrum* (6), previously described as *Lepidocarpon* (27, 31). This species is based on ovoid fossils which are very abundant in certain of the Iowa mines. The wall of the "seed" is interpreted as a multilayered, vascularized sporangium ("nucellus") enclosing a single large megaspore. Associated with it are other fossils which are believed to represent a different (abnormal?) growth stage. These contain an irregularly proliferated vascularized tissue, originally interpreted as a sporeling and considered by the present writer (6) as an aposporous growth of the parenchymatous layer of the nucellus or a gemma-type reproductive tissue. It is suggested that *Nucellangium* may be a primitive cordaitean "seed", and comparisons are drawn with certain *Cardiocarpus* type compression fossils.

CONCLUSION

Interest in American coal-ball floras has increased perceptibly in the past few years, and some attempts have been made in this brief review to point out current lines of research and what may be expected during the next decade or two. Two other topics might have been considered, namely, a detailed comparison with the European fossils, and a stratigraphic compilation of the Ameri-

can floras. Such considerations would, I believe, be premature at present. We have many genera and a considerable number of species in common with those found in the European coal balls; it is also evident that there were certain elements in the American scene that did not occur on the other side of the Atlantic. Such a comparative picture is, however, only beginning to crystallize. The tendency to date has been to describe those fossils that are new, especially conspicuous and well preserved, and we are still a long way from the end of this "cream-skimming" stage. With continuation of such studies, which are now in progress in four or five laboratories, and the integration of botanical and geological interests, we should ultimately be able to work out a very interesting picture of the sequence of Upper Carboniferous floras and contribute notably to an understanding of the evolution of certain pteridophytic groups and the early seed plants.

ACKNOWLEDGMENTS

It is with sincere gratitude that I acknowledge the aid of the following colleagues in preparing this review: Dr. Fredda D. Reed and Dr. J. H. Hoskins for biographical data on Professor Noé; Dr. Sergius Mamay for assisting with the discussion of the coenopterids; Dr. James M. Schopf and Dr. R. M. Kosanke for much aid in the field as well as for information bearing on the stratigraphy of the coal beds.

ADDENDA

In the few months that have passed since this review was submitted for publication, several contributions have appeared in print to which I should like to make at least brief reference.

Baxter (17*a*) has described a new genus of sphenophyll cones from Indiana which adds to our rapidly growing knowledge of that group. His material is also notable from the standpoint of the quality of preservation, well-preserved nuclei being present in the spores. A particularly noteworthy contribution is Mamay's (64*a*) detailed treatment of the fructifications referred to the Marattiaceae and coenopterid ferns; several new species are described, and a thorough consideration is given to the evolution and relationships of these fossils. Many of these have been overlooked or neglected in the past probably due to their small size. The De-

cember issue of the Bulletin of the Torrey Botanical Club (64*b*) contains the results of our study of the vascular anatomy of *Botryopteris*. Traverse (99*a*) has presented an admirable discussion of the anatomy of Cordaitalean stems from Iowa coal balls.

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